No. 15-1356

UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT

ROCHE DIAGNOSTICS OPERATIONS, INC.
and CORANGE INTERNATIONAL LIMITED,
Plaintiffs-Appellants,

v.

LIFESCAN INCORPORATED,
Defendant-Appellee,
and

NOVA BIOMEDICAL CORPORATION,
Defendant-Appellee.

Appeal from the United States District Court for the District of Delaware in case no. 07-CV-0753, United States District Judge Richard G. Andrews

BRIEF OF APPELLANTS ROCHE DIAGNOSTICS OPERATIONS, INC. AND CORANGE INTERNATIONAL LIMITED

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April 21, 2015

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ROCHE DIAGNOSTICS OPERATIONS v. LIFESCAN INCORPORATED

No. 15-1356

CERTIFICATE OF INTEREST

Pursuant to Federal Circuit Rules 27(a)(7) and 47.4, counsel for Plaintiffs-Appellants Roche Diagnostics Operations, Inc. and Corange International Limited (n/k/a Roche Operations Ltd.) certifies the following:

1. The full name of every party or amicus represented by me is:

Roche Diagnostics Operations, Inc. and Corange International Limited (n/k/a Roche Operations Ltd.)

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is:

Not Applicable

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party or amicus curiae represented by me are:

Appellants, Roche Diagnostics Operations, Inc. and Corange International Limited (n/k/a Roche Operations Ltd.), are wholly-owned by Roche Holding Ltd, which is publicly traded. Novartis AG, through a wholly-owned subsidiary, owns more than 10% of Roche Holding Ltd.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or that are expected to appear in this court are:

McDONNELL BOEHNEN HULBERT & BERGHOFF LLP: Daniel A. Boehnen, Grantland G. Drutchas, Richard A. Machonkin, Sean M. Sullivan, Christina L. Brown, Patrick G. Gattari, Christopher M. Cavan, Paul S. Tully, Gary E. Hood, Nicole A. Keenan, Eric R. Moran, Paula S. Fritsch, Sherri L. Oslick, Jeffrey P. Armstrong, Benjamin R. Huber, James V. DeGiulio;

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Respectfully submitted,

Dated: April 21, 2015

By: /s/ Grantland G. Drutchas

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STATEMENT OF RELATED CASES

There is currently no appeal in or from this civil action before this or any other appellate court. No other lawsuit known to counsel to be pending in this or any other court will directly affect or be directly affected by this Court's decision in the pending appeal. This case was previously appealed to this Court as *Roche Diagnostics v. Lifescan*, Appeal Nos. 2010-1439 and 2010-1539. Those appeals resulted in an Opinion by Chief Judge Prost that issued on January 25, 2012.

JURISDICTIONAL STATEMENT

The jurisdiction of the District Court over this action arises under 28 U.S.C. §§ 1331 and 1338(a). This Court has jurisdiction over the final judgment pursuant to 28 U.S.C. § 1295(a)(1). The appeal is from a final judgment entered on January 16, 2015. Appellants timely filed a Notice of Appeal on February 13, 2015.

STATEMENT OF THE ISSUES

1. Whether the District Court erred in limiting the "electrode" claim terms to the preferred embodiments – "microelectrodes having a width of 15 μm up to approximately 100 μm" – by ignoring the ordinary meaning of "microelectrode," disregarding the role of diffusion disclosed in the intrinsic

evidence, dismissing Examples 3-5 of the '146 patent, and performing a faulty "enablement lite" analysis, even though the ordinary meaning and the intrinsic evidence establish that microelectrodes have widths of up to approximately 1,000 μm.

2. Whether the District Court erred procedurally and substantively in its application of the Third Circuit's standard for reconsideration to the District Court's interlocutory order on claim construction as opposed to the rolling claim construction standard applied by the Federal Circuit.

STATEMENT OF THE CASE

On November 21, 2007, Roche Diagnostics Operations, Inc. and Corange International Limited (n/k/a Roche Operations Ltd.) (collectively, "Roche") filed a Complaint against LifeScan, Incorporated ("LifeScan") and Nova Biomedical Corporation ("Nova")[1] (and several former defendants) alleging infringement of two Roche patents – U.S. Patent Nos. 7,276,146 and 7,276,147 (the "'146 patent" and the "'147 patent"; collectively, the "patents-in-suit") – covering certain blood glucose monitoring technologies.

The District Court phased fact discovery (i) starting with fact discovery on infringement issues, (ii) followed by the *Markman* briefing and hearing

[1] LifeScan and Nova are referred to collectively as "Defendants-Appellees."

("Pre-Remand *Markman*"), and then (iii) fact discovery on the validity issues. Expert discovery on all issues took place following the close of validity fact discovery. (A2047-2051; A2040-2041). While the parties were awaiting the District Court's *Markman* decision, they completed fact discovery on validity and expert discovery on all issues. Defendants-Appellees filed their motions for summary judgment of non-infringement on August 28, 2009.

The District Court issued its Memorandum Opinion ("Pre-Remand Opinion") and Order on claim construction on September 15 and 16, 2009, respectively, while summary judgment briefing was still underway. (A6-31; A3-5). With respect to the "electrode" claim terms ("working electrode," "counter electrode," and "reference electrode"), the District Court imposed a limitation expressly taken from descriptions of the preferred embodiments:

> Rather, the specification explains that the "preferred dimension" is "from 15 or 20 or 25 μm, up to about 100 μm...." Accordingly, the Court will construe these "microelectrode" terms to refer to microelectrodes having a width of 15 μm up to approximately 100 μm.

(A14-15 (citation omitted)).

Concurrently with the summary judgment briefing, and consistent with D. Del. Local Rule 7.1.5, Roche timely moved for reconsideration of the District Court's construction of the "electrode" claim terms on September 29, 2009, citing legal errors with the District Court's construction and additional evidence

gleaned through continuing fact and expert discovery. Defendants-Appellees filed a joint opposition to Roche's motion for reconsideration on October 14, 2009. Thereafter, Roche sought leave to file a reply brief in support of the motion for reconsideration. Roche's motion for reconsideration of the claim construction decision was initially heard on January 14, 2010, with additional briefing requested by the Court. At a hearing on January 21, 2010, the District Court declined to revise its claim construction ruling, but acknowledged that its claim interpretation was problematic:

> I've looked at the paper [briefing] on reconsideration. It's a great point for the Federal Circuit, and I actually think you might have a point. But it will be interesting to see what they say. So we will be moving ahead with that Rule 54 judgment.

(A35 at 5:24-6:6).[2] The District Court ordered the parties to file a motion for entry of a Rule 54(b) judgment and proposed order.

On July 27, 2010, the District Court granted Defendants-Appellees' motions for summary judgment of non-infringement, and Roche's motion for a Rule 54(b) judgment on its patent infringement claims. Roche filed its Notice of

[2] Pages and line numbers to transcripts are cited as "[page]:[line]." Column and line numbers of the patents-in-suit (A39-79 and A80-106) and other patents in the record are cited herein as "[column]:[line]."

Appeal that same day.[3]

Roche's appeal was argued on November 7, 2011. *See* Appeal No. 10-1439. On January 25, 2012, this Court vacated the judgment of non-infringement and remanded the case to the District Court for further proceedings, with questions for the District Court to address on remand.

On May 4, 2012, newly-assigned District Court Judge Andrews ordered additional claim construction briefing and scheduled a *Markman* hearing directed specifically to "hear[ing] argument on claim construction of the term 'electrode'" ("Post-Remand *Markman*"). (A26210). Judge Andrews invited "the parties to supplement the record as they saw fit" (A1.15), and both sides submitted supplemental evidence on the definition of the term "electrode," including declarations of both sides' experts, Professors Weber and Higson, and numerous additional exhibits not presented with their original *Markman* briefs.

The Post-Remand *Markman* hearing was held on September 5, 2012. At the hearing, the District Court also raised *sua sponte* the issue whether Defendants-Appellees maintained an objection on appeal to the consideration of all of the evidence and arguments regarding claim construction, including that

[3] Nova's cross-appeal on its trade secret misappropriation counterclaims was denied, with this Court affirming the District Court's and the jury's resolution of Nova's counterclaims.

adduced only on reconsideration. It ordered the parties to brief (1) whether Roche's motion for reconsideration was procedurally appropriate, and (2) if so, whether Defendants-Appellees waived any procedural objections to Roche's claim construction arguments by not addressing them on appeal.

Although Defendants-Appellees represented to the District Court that "we were very clear in our briefs…that they were out of time on the reconsideration motion and that the reconsideration motion was a problem," (A28989:20-24), Defendants-Appellees were unable to identify anywhere in the briefs (or oral argument, for that matter) where Defendants-Appellees raised any objection to such evidence or arguments. As the District Court found on remand, Defendants-Appellees' "response was underwhelming" because the "provided citations do not show any argument at all about reconsideration being procedurally improper." (A1.14). To the contrary, Defendants-Appellees themselves relied on evidence that was first addressed in the reconsideration proceeding in support of their claim construction position. Defendants-Appellees relied on this new evidence in their reconsideration briefing, in the prior appeal, and in the Post-Remand *Markman* hearing. As this Court found, "Nova and Lifescan do not dispute on appeal, however, that Roche's argument should be addressed on the merits." *Roche Diagnostics Operations, Inc. v. LifeScan Inc*., 452 Fed. Appx. 989, 994 (Fed. Cir. 2012).

On December 5, 2014, the District Court issued an Order and Memorandum Opinion ("Post-Remand Opinion") (1) confirming the original claim construction on the "electrode" terms by once again importing a limitation from the preferred embodiments and ignoring intrinsic evidence that established electrodes up to 1,000 μm in width constitute microelectrodes; (2) responding to the questions posed by this Court in the previous appeal; and (3) addressing the reconsideration issues. (A1.5; A1.6-1.20).

STATEMENT OF THE FACTS

I. Technical Summary of the Claimed Inventions

The patents-in-suit teach electrochemical methods for determining glucose concentration in a blood sample using a specifically defined disposable biosensor strip. The patents-in-suit teach and claim a method for measuring glucose in blood using a biosensor strip that accommodates a small sample volume (about 1 μL or less) and provides a readout of the glucose concentration within about 10 seconds of sample detection. (A70 at claim 31; A102 at claim 36). Capillary action draws the blood into a capillary chamber in the biosensor strip, which contains a working electrode and a counter electrode. When the blood sample fills the capillary chamber, the electrodes detect the sample and the test time begins.

A chemical reagent positioned over the working electrode within the capillary chamber is solubilized by the sample, and reacts with glucose in the blood to produce an electroactive reaction product. The reaction produces an electrical current that is measured and correlated to a concentration of glucose in the blood sample. Within 10 seconds after the sample is detected, the sensor provides a readout of the glucose concentration.

Dependent claims specify smaller sample volumes or shorter test times or additional structural or functional aspects of the claimed inventions, *e.g.*, claims 16 and 48 of the '146 patent and claims 19 and 53 of the '147 patent ("capillary chamber has a depth of 25-200 μm"). (A69-70; A101-102).

II. The State of the Art at the Time of the Invention

Prior to the invention described and claimed in the patents-in-suit, electrochemical glucose sensing systems required relatively large blood sample volumes (on the order of 2.5-4 μL) and/or a relatively long period of time (up to a minute) to produce a glucose reading. (A251, 1:19-25; A260, Table 1; A278, 11:1-10). The sample sizes in the art at the time of the invention required users to make relatively large, deep cuts in their fingertips to obtain enough blood to run the test and could require re-testing. (A1270, 2:3-5). The longer test times required by these prior art systems were also significant disincentives to users.

III. Mr. Wilsey's Invention

In 1996 and early 1997, a Roche scientist, Chris Wilsey, began working on a glucose sensor that would yield a reproducible test using much smaller sample volumes and significantly shorter test times. He initially achieved this result in part by adapting printed circuit board manufacturing techniques to glucose biosensor electrodes and by designing a capillary chamber that was extremely small and directed blood flow to a very precise location of chemical reagent and electrode structures. (A60, 12:27-30; A62-63, 16:16-17:52; A95, 11:17-20; A97, 15:5-16:41). He also found, counter-intuitively, that he could produce faster test times by reducing reagent concentrations. (A61, 13:47-56; A95, 12:37-46). Normally, persons of skill in the art would have expected that faster test times required increasing, not decreasing, reagent concentration in order to speed up the chemical reactions. (*See, e.g.,* A275, 6:4-6 ("The more enzyme added, the shorter the time period for completion of the reaction."); A611-612).

Mr. Wilsey was the first to measure the blood glucose concentration in substantially less time after sample detection than prior art systems, including providing a result within about 4 seconds of detection, with sub-microliter sample volumes. (*See, e.g.*, A55, 2:51-61; A60, 12:46-49; A62, 15:21-32).

IV. Prosecution History of the Patents-In-Suit

The patents-in-suit both claim priority to the same provisional application, Serial No. 60/332,411, filed November 16, 2001 (the "Provisional Application"). The '146 patent issued from Application Serial No. 10/264,891, filed October 4, 2002 ("'891/'146 application"). A second application, Serial No. 10/264,785 was filed the same day; the '147 patent issued from a continuation of that application, Application Serial No. 10/382,322, which was filed on March 3, 2003 (the "'322/'147 application"). Both patents-in-suit issued on October 2, 2007.

During the prosecution of both applications, the applicant filed an inventor declaration ("Wilsey Declaration") in which Mr. Wilsey described some of the milestones of his invention and included, as Exhibit C, a 1997 review of data from his laboratory in a document entitled "Excalibur Data Review" ("Excalibur Report"). (A906-1018). The Excalibur Report summarized as of March 27, 1997, the work performed by Mr. Wilsey and his team on microelectrode biosensor strips with electrodes as wide as 1 millimeter (1,000 μm).

The patent specifications and their prosecution histories (including the Excalibur Report) disclose microelectrodes of various dimensions, including:

- Mr. Wilsey's "box-hook microelectrodes" (*See* A935; A941; A948; A970-971) which were 1 mm, or 1,000 μm, wide. (A926; Example 3 of the '146 patent (A67-68)).
- Mr. Wilsey's "3-finger microelectrode (1 WE & 2 CE)" (A936), having three 500 μm-wide working and counter electrodes. (A927; Example 4 of the '146 patent (A68)).
- Mr. Wilsey's "5-finger microelectrode (2 WE & 3 CE)" (A937), having four 300 μm-wide working and counter electrodes, and one 225 μm-wide counter electrode. (A928; similar to Example 5 of the '146 patent (A68)).

These three microelectrodes were explicitly described in the Wilsey Declaration submitted in the prosecution histories of both patent applications and in the specification of the '146 patent, but were not set forth in the Provisional Application or the '147 patent. As the District Court noted, however, "[t]he parties agree that 'electrode' should be construed the same way in the '146 and '147 patents." (A1.17).

V. The Defendants-Appellees' Accused Products

In use, Defendants-Appellees' accused test strips and meters have or perform each limitation found in the claims as construed by the District Court, with one exception: the widths of the electrodes in Defendants-Appellees'

accused products are wider than the upper size limit imposed by the District Court from the preferred embodiments ("up to approximately 100 μm"), but well under 1,000 μm, the ordinary understanding of a microelectrode. Under the District Court's claim construction, Defendants-Appellees' accused products do not infringe the asserted claims.

SUMMARY OF THE ARGUMENT

In construing the "electrode" claim terms, the District Court concluded that the applicant had disavowed macroelectrodes during the prosecution of one of the underlying applications, leaving the claims limited to microelectrodes. But that begs the question: what distinguishes microelectrodes from macroelectrodes? The ***macro***electrodes the applicant distinguished during prosecution were at least 1,600 μm in width, much larger than the maximum width of approximately 100 μm that the District Court imposed. Further, under the District Court's incorrect construction, the electrodes disclosed in Examples 3-5 of the '146 patent and in the Excalibur Report are indisputably not microelectrodes and would therefore be outside the scope of the claims. Yet such intrinsic evidence clearly and unambiguously describes these electrodes as microelectrodes, and also repeatedly describes the 100 μm width as simply preferred.

The ordinary and customary meaning of "microelectrode," inherent from the use of the prefix "micro," is any electrode measured in micrometers or μm (up to 1,000 μm). The applicant explicitly stated in the specification that it did ***not*** intend to provide any unique lexicography, noting that "microelectrodes … are understood in the electronic and biosensor arts." (A56, 4:29-31; A91, 4:10-12). The intrinsic evidence also describes electrodes up to 1,000 μm in width as microelectrodes and includes broad teachings of "larger-dimensioned electrodes."

Indeed, the District Court's construction is inconsistent with, and narrower than, the description of a microelectrode set forth in the original claims 1 and 2 of the Provisional Application, which are part of the original disclosure. Furthermore, the Excalibur Report, submitted in the prosecution of both patents-in-suit, expressly and repeatedly describes electrodes larger than 100 μm and up to 1,000 μm as "microelectrodes."

This ordinary meaning and intrinsic evidence is thus consistent with the leading textbook, technical dictionary, and encyclopedia in the field, which universally define a microelectrode as an electrode having a width of up to 1 mm, or 1,000 μm.

Nevertheless, both pre- and post-remand, the District Court ignored the ordinary and customary meaning, and ignored or rejected all disclosures of

electrodes of up to 1,000 μm in width in the intrinsic evidence. Instead, at Defendants-Appellees' urging, it looked to preferred embodiments in the Detailed Description to discern a size definition for microelectrode.

The District Court's Post-Remand Opinion pinned the entire construction on a single sentence in one paragraph of the Detailed Description:

> It is also understood that some electrode configurations can cause diffusion to take place by a mix of planar and non-planar paths, in which case the electrodes can be considered a [microelectrode] array, especially if the diffusion occurs predominantly (e.g., greater than 50%) according to a non-planar path, or if the size of the electrodes is less than 100 μm, e.g., less than 50 μm.

(A1.16, citing A91, 4:23-29).

Based on this one sentence, the District Court found that a "microelectrode" required one of the two "situations" identified therein:

> The specification, thus, seems to indicate that an electrode might be characterized as a microelectrode in one of two situations: (1) where there is greater than 50% non-planar diffusion, or (2) where the electrode has a width less than 100 μm.

(A1.16). Inexplicably, the District Court ignored (a) the qualifier "especially" that modifies these "situations," meaning that they are merely preferred

embodiments,[4] and (b) the primary phrase in that sentence which states that "electrode configurations [that] can cause diffusion to take place by a mix of planar and non-planar paths … can be considered a micro-electrode array…." (A56, 4:43-45; A91, 4:24-26). Equally importantly, the District Court also disregarded the topic sentence of the same paragraph, which indicates on its face that the applicant did not intend to be his own lexicographer. "Micro-electrodes, as distinguished from other electrodes generally, are understood in the electronic and biosensor arts." (A91, 4:10-12; A56, 4:29-31). The District Court's imposition of a dimension from the preferred embodiments is a clear violation of the cardinal rule against limiting claims to the preferred embodiments and is legal error on its face. The District Court also ignored or disregarded language in the specification that clearly describes microelectrodes more broadly.

As part of its remand in the first appeal, this Court instructed the District Court to consider "what degree of non-planar diffusion justifies characterizing an electrode as a micro-electrode." *Roche*, 452 Fed. Appx. at 995. The District

[4] This Court previously noted that the passages from the specifications of the patents-in-suit, including language such as "preferred dimensions," "especially," or "typically," are "rather persuasively, in our view," merely "non-limiting description[s] of a preferred embodiment." *Roche*, 452 Fed. Appx. at 995.

Court's construction applies one of the preferred embodiments described above, i.e., where there is greater than 50% non-planar diffusion, (A56, 4:45-47; A91, 4:26-28) and ignores the primary phrase in that final sentence that says that ***any*** electrode that exhibits "a mix of planar and non-planar [diffusion] … can be considered a micro-electrode array." (A56, 4:43-45; A91, 4:24-26). Nothing in the record supports the proposition that an electrode must be less than about 100 μm to exhibit such a mix of planar and non-planar diffusion. To the contrary, all evidence – including Defendants-Appellees' experts' admissions – confirms that an electrode of up to 1,000 μm in width ***will*** have such a mix of planar and non-planar diffusion. Ultimately, the District Court simply chose to ignore diffusion on remand: "There is some difficulty in converting the first [diffusion] characterization into a size, as [the specification] gives no basis for doing so." (A1.16). Regardless, diffusion does not impose or require a 100 μm width limitation.

The District Court also improperly ignored Examples 3-5 of the '146 patent. As the District Court noted, the parties agree that "electrode" should be construed the same way in both patents-in-suit. (A1.17). The District Court's sole rationale on remand for rejecting these Examples was its conclusion that they are unclaimed embodiments. (A1.16-1.17). While those Examples describe experiments performed using glucose solution, not blood, the type of

solution used does not dictate whether the 1,000, 500, and 300 μm wide electrodes used in those experiments are microelectrodes. Examples 3-5 are instructive regarding the widths of microelectrodes, regardless of whether they tested glucose solution or blood. The District Court also failed to answer this Court's essential question as to whether Defendants-Appellees' "fast fill" argument is relevant to the claims. As a matter of law it is not.

The District Court also improperly disregarded dependent claims directed to capillary chambers having depths of 25-200 μm. The prosecution history establishes that these claims were specifically added by amendment, with appropriate citations detailing written description support. The District Court erred when it concluded that a claim with such a depth limitation, claim 48, was not enabled and could be ignored for claim construction purposes. The District Court applied an unprecedented "enablement lite" analysis without consideration of any of the underlying factors. Instead, the District Court improperly conflated the Examiner's early written description objection with enablement, and failed to give any deference to the Examiner's allowance of the claims. (A1.18-1.19). Under a proper enablement analysis, claim 48 is clearly enabled, and thus is relevant to construction of the "electrode" terms. Moreover, the Defendants-Appellees never offered any other evidence challenging the enablement of the claims of the patents-in-suit.

With respect to extrinsic evidence, the District Court was asked to determine if and to what extent any of the additional evidence should be admitted into evidence. In response, the District Court found it was "in the interest of justice to allow the parties to supplement the record as they saw fit." (A1.15). The District Court, however, did not rely on such extrinsic evidence, holding that "Roche's dictionary references do not trump the intrinsic evidence already considered by this Court." (A1.20). This holding reflects a fundamental legal error as to the content of the intrinsic evidence as a whole and the ordinary meaning of the term "microelectrode." Indeed, it is undisputed that the leading electrochemistry textbook, the leading technical dictionary, and the leading encyclopedia of chemical technology all confirm that microelectrodes have widths of up to 1 mm, or 1,000 μm, which is fully consistent with the intrinsic evidence and ordinary meaning.

Finally, the District Court also erred in its application of Third Circuit standards for reconsideration to an interlocutory order on claim construction. Such interlocutory orders are, as this Court has held, subject to a rolling claim construction in which the District Court can revisit and alter its interpretation of the claim terms as its understanding of the technology evolves. This is particularly appropriate given the situation here, where the Pre-Remand *Markman* occurred before any validity or any expert discovery. Even applying

the Third Circuit reconsideration standards, the District Court erred as a matter of law. Moreover, some of the evidence cited above, including the admissions by Defendants-Appellees' experts that electrodes over 100 μm in width exhibit a mix of planar and non-planar diffusion, was not available at the time of the Pre-Remand *Markman* hearing. Finally, Defendants-Appellees waived any procedural objection to reconsideration by failing to raise those objections in the first appeal and by relying on new evidence on reconsideration, on remand, and on appeal.

The correct construction of the "electrode" terms is "microelectrodes having a width of 15 μm up to approximately 1,000 μm." Therefore, the District Court's claim construction Order that ignores much of the intrinsic evidence should be reversed as a matter of law, the District Court's decisions on Defendants-Appellees' motions for summary judgment of non-infringement should be vacated as predicated on an erroneous construction, and this case should be remanded for further proceedings.

ARGUMENT

I. Introduction

The question before this Court is whether the "electrode" claim terms mean an electrode up to approximately 1,000 μm in width or 100 μm in width.

In its Pre-Remand Opinion, the District Court concluded that "electrode" as used in the patent claims means "microelectrode" because the specification refers to "microelectrodes" as aspects of the invention and because "the patentee distinguished the prior art, in part, on the basis of its use of macroelectrodes as opposed to microelectrodes." (A14). Roche did not dispute that finding in the first appeal and is not disputing it now because the finding has no practical impact on the issue before this Court. As discussed below, the prior art references that were the subject of the purported disavowal disclose "macroelectrodes" having widths of 1,600 μm or greater, much greater than the width limitations that either side proposed for the proper construction of the "electrode" terms.

Thus, this Court must determine *de novo* whether to impose a 1,000 μm or a 100 μm width limitation on the claimed microelectrodes.

The ordinary and customary meaning of microelectrode is any electrode measured in micrometers (μm), up to 1,000 μm. This meaning is inherent from the use of the prefix "micro." This meaning is also consistent with the intrinsic evidence, the leading technical treatises, scientific articles and patents, and common sense. Like the ordinary meaning and the intrinsic evidence, the leading textbook, technical dictionary, and technical encyclopedia in the field expressly and universally confirm that the accepted technical definition of a

microelectrode is an electrode having a width of up to 1,000 μm: *Electrochemical Methods, Fundamentals and Applications*, by Bard & Faulkner (2nd Ed. 2001) (microelectrodes having "micrometer" dimensions and "a 1-mm diameter microelectrode"); *Electrochemical Dictionary* (2008) ("Microelectrode – Electrode with a characteristic dimension ranging from 25 μm up to 1 mm."); *The Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 9 (4th Ed. 1994) and Vol. 9 (5th Ed. 2005) ("Small, referring to the diameter of the electrode, is about a millimeter for microelectrodes, or perhaps only a few micrometers for ultramicroelectrodes…"). Thus, construing microelectrode to mean a different or smaller width than 1,000 μm would be to disregard the ordinary and customary meaning of the term, not to interpret it.

Under this Court's case law, a term must be given its ordinary meaning unless the applicant has clearly and affirmatively adopted a contrary lexicography. Here, there was no express attempt to limit or alter the ordinary meaning of microelectrode. Moreover, the uses of "microelectrode" – a term whose purpose is to denote electrodes up to 1,000 μm – throughout the intrinsic record precludes any plausible argument that electrodes of some narrower size range are the only electrodes encompassed by the claims. Providing preferred examples of microelectrodes that have smaller sizes cannot in any way narrow the meaning of microelectrode itself. Such a narrowing "interpretation" of the

term would not be an interpretation of it, but a blatant disregard of the word's actual meaning. And a construction of "microelectrode" that excludes all microelectrodes between 100 and 1,000 µm – 90% of the possible range – is plainly an improper limitation that goes well beyond a reasonable interpretation of the word. As shown in Fig. 1 at right, the specifications and the intrinsic evidence viewed as a whole are fully consistent with the full scope of the ordinary understanding of microelectrode, namely up to approximately 1,000 µm.

Roche's motion for reconsideration focused on the legal errors committed in the District Court's Pre-Remand Opinion. After considering the arguments pre-remand, the District Court acknowledged

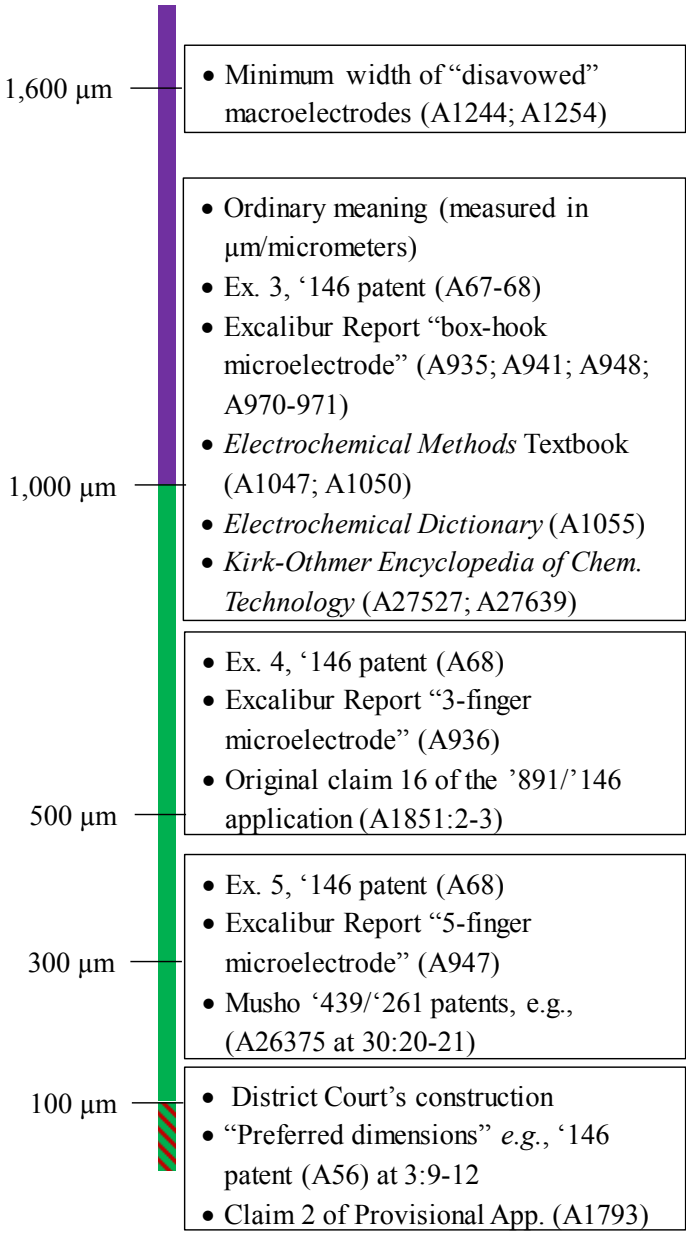

Fig. 1

on reconsideration that its construction was problematic but nevertheless refused to revise its claim construction. (A35, 5:24-6:6). Post-remand, the District Court invited supplementation of the record by the parties, and attempted to address the specific issues this Court directed it to address on remand. But it used the same faulty logic to arrive at the same erroneous construction. Neither opinion addressed the broad statements about electrodes in the specification, the claims as filed in the original application, the description of microelectrodes of up to 1,000 μm in the prosecution history, or any other intrinsic or extrinsic evidence that confirms that "microelectrode" should be construed consistent with its ordinary and customary meaning, namely up to approximately 1,000 μm.

II. Standard of Review

Issue 1: Claim Construction: Because the District Court relied solely on the intrinsic evidence to reach its decision on the construction of the "electrode" claim terms, the Court's claim construction is reviewed *de novo* under *Teva Pharms. USA, Inc., v. Sandoz, Inc.,*—— U.S. —— (2014), 135 S.Ct. 831, 841 (2015); *Mobilemedia Ideas LLC v. Apple Inc*., 780 F.3d 1159, 1169 (Fed. Cir. 2015). The District Court's single reference to and rejection of the undisputed technical dictionary, textbook and other reference works is legal error as well,

because the District Court's treatment of those materials was based on its incorrect interpretation of the intrinsic evidence.

Issue 2: *Markman* Reconsideration: Although motions for reconsideration by a district court are generally reviewed under the standard of review used by the governing regional circuit, *Minton v. NASD, Inc.*, 336 F.3d 1373, 1378-79 (Fed. Cir. 2003), where, as here, the question pertains uniquely to patent law (the revision of an interlocutory *Markman* order), this Court applies its own law. *See Midwest Indus., Inc. v. Karavan Trailers, Inc.,* 175 F.3d 1356, 1359 (Fed. Cir. 1999) (*en banc* in relevant part). Regardless, however, under either Third Circuit or Federal Circuit law, the question of whether the District Court applied the correct legal standard is a question of law reviewed by this Court *de novo*. *See Pharmacia & Upjohn Co. v. Mylan Pharm., Inc.*, 182 F.3d 1356, 1359 (Fed. Cir. 1999); *Robeson Indus. Corp. v. Hartford Acc.& Indem. Co.*, 178 F.3d 160, 164 (3d Cir. 1999). To the extent that the Third Circuit standard on reconsideration applies, the reconsideration decision is reviewed for abuse of discretion and should be reversed where it is deemed "arbitrary, irrational, or contrary to the law." *Castro v. Att'y Gen.*, 671 F.3d 356, 365 (3d Cir. 2012) (quotation marks omitted).

III. The District Court Improperly Limited the Claims to the Electrode Widths of the Preferred Embodiments

In essentially adopting Defendants-Appellees' proposed construction, the District Court improperly limited the claims to the electrode widths of the preferred embodiments despite incontrovertible intrinsic evidence to the contrary.

A. The District Courts' Opinions Establish on Their Face that It Improperly Limited the Claims to the Electrode Widths of the Preferred Embodiments

In its Pre-Remand Opinion, the District Court readily acknowledged that it limited the claims to the preferred embodiments:

> However, the specification, unlike Defendants' proposed construction, does not describe the upper limit of the range as a strict cutoff. Rather, the specification explains that the 'preferred dimension' is 'from 15 or 20 or 25 μm, up to about 100 μm ....' (*Id.* at 3:7-15.) Accordingly, the Court will construe these 'microelectrode' terms to refer to microelectrodes having a width of 15 μm up to approximately 100 μm. This construction illuminates the size of a microelectrode to one of skill in the art without improperly excluding microelectrodes that are slightly larger than the preferred dimensions.

(A14-15). As such, the District Court simply and impermissibly engrafted the preferred dimensions for the microelectrodes from the specification into the claims.[5]

[5] Although the District Court's Pre-Remand Opinion indicated that the range it used is "slightly larger than the preferred dimensions," it is hard to understand

In its Post-Remand Opinion, the District Court applied the same faulty logic:

> Specifically, the specification states:
>
> > It is also understood that some electrode configurations can cause diffusion to take place by a mix of planar and non-planar paths, in which case the electrodes can be considered a [microelectrode] array, ***especially*** if the diffusion occurs predominantly (e.g., greater than 50%) according to a non-planar path, or if the size of the electrodes is less than 100 μm, e.g., less than 50 μm.
>
> '147 patent at col.4 ll.23-29. The specification, thus, seems to indicate that an electrode might be characterized as a microelectrode in one of two situations: (1) where there is greater than 50% non-planar diffusion, or (2) where the electrode has a width less than 100 μm. … The second characterization supports the Court's construction.

(A1.16) (emphasis added). That is, the District Court ignored the rest of the specification and the prosecution history, and discerned a definition from these preferred, "especial" situations.

The District Court's pre- and post-remand construction reading the "about [or approximately] 100 μm" width limitation into the claims from the preferred embodiments contradicts the holdings from a long line of this Court's cases.

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how "up to ***approximately*** 100 μm" is "slightly larger" than "up to ***about*** 100 μm." (A15) (emphases added). Indeed, elsewhere in the same opinion, the District Court "concludes that the term 'about' should simply be given its ordinary and accepted meaning of 'approximately.'" (A27).

See, e.g., Kara Tech. Inc. v. Stamps.com Inc., 582 F.3d 1341, 1348 (Fed. Cir. 2009) ("we will not limit him to his preferred embodiment or import a limitation from the specification into the claims"); *Phillips v. AWH Corp.*, 415 F.3d 1303, 1323 (Fed. Cir. 2005) (*en banc*) ("[W]e have repeatedly warned against confining the claims to those embodiments"); *SRI Int'l v. Matsushita Elec. Corp.,* 775 F.2d 1107, 1121 (Fed. Cir. 1985) (if claims were limited to specification-described embodiments, "there would be no need for claims").

This is not a case where the patentee has acted as its own lexicographer, "clearly set[ting] forth a definition of the disputed claim term other than its plain and ordinary meaning." *Thorner v. Sony Computer Entm't Am. LLC*, 669 F.3d 1362, 1365 (Fed. Cir. 2012) (quotation marks omitted). Thus, the Court is to "consider the specification as a whole, and to read all portions of the written description, if possible, in a manner that renders the patent internally consistent." *Pfizer Inc. v. Teva Pharms. USA, Inc.,* 429 F.3d 1364, 1373 (Fed. Cir. 2005) (quotation marks omitted). This approach requires a careful "contextual analysis" that is wholly missing from the District Court's pre- and post-remand construction. *See Dealertrack, Inc. v. Huber*, 674 F.3d 1315, 1326 (Fed. Cir. 2012).

In this case, each and every reference in the Provisional Application (and the patents-in-suit) to numerical electrode sizes of 100 μm or less contains

qualifying language indicating that such a range is only preferred, including, "preferred dimensions,"[6] "especially,"[7] or "typically."[8] As this Court noted, these passages are "rather persuasively, in our view" merely non-limiting descriptions of a preferred embodiment of the claimed invention. *Roche*, 452 Fed. Appx. at 995. Neither the specification nor any other intrinsic evidence provides any justification for limiting the claims to this preferred range. Nor does the specification or the prosecution history contain any disavowal of the full scope of "microelectrode," which includes electrodes up to 1,000 μm in width.

B. The Intrinsic Evidence Precludes Limiting the Claims to the Preferred Embodiments

1. The Broad Language Throughout the Specifications Regarding Electrode Size Precludes Limiting Microelectrodes to Approximately 100 μm in Width

Throughout the specifications of the Provisional Application and the patents-in-suit, the applicant used broad, open-ended language to describe the invention as including electrodes in general and larger-dimensioned microelectrodes in particular, both of which are discussed without any mention

[6] (A1763:5-8; A1766:1-4; A1779:31-32; A56, 3:9-15; A57, 5:33-37; A62, 15:49-51; A91, 3:9-15; A92, 5:14-18; A96, 14:38-40).
[7] (A1766:5-8; A56, 4:42-48; A91, 4:23-29).
[8] (A1780:8-12; A62, 15:63-66; A96, 14:52-55).

of any specific size limitations. In limiting the claims to the preferred microelectrodes of "up to approximately 100 μm" in width, the District Court ignored all of these passages. Further, as discussed in more detail in Section III.D below, it also improperly disregarded the electrodes in Examples 3-5 of the '146 patent.

Rather than providing a unique definition for "microelectrode," the specification as a whole indicates that the applicant intended to apply the full scope of "microelectrode" as it would generally be understood by those skilled in the art. Indeed, the topic sentence for the paragraph that the District Court used to support its construction on remand, the third paragraph of the Detailed Description, states explicitly that, "Micro-electrodes, as distinguished from other electrodes generally, are understood in the electronic and biosensor arts." (A1764:13-14; A56, 4:29-31; A91, 4:10-12). This language directly contradicts any clear expression of an attempt to uniquely define the term under *Thorner*.

Other passages throughout the specifications confirm that the invention makes broad use of electrodes regardless of whether their dimensions are within the preferred range. For example, the "Summary of the Invention" section states:

> More particularly, ***the present invention*** comprises a method for determining the concentration of glucose in a blood sample. The method utilizes a disposable test strip having a capillary-fill chamber including a working ***electrode*** and a counter and/or reference ***electrode*** and a reagent.

(A56, 3:40-44 (emphasis added); *see also* A91, 3:41-45).

Other broad, open-ended language describes the electrodes of the invention as including any dimension that can provide useful or advantageous capabilities:

> ***The electrodes and their components can be of dimensions, meaning the width of the electrode components*** as well as the separation between components, ***that can provide an array with useful properties, e.g., useful or advantageous capabilities with respect to contacting a substance or measuring electrical properties***.

(A1765:26-29 (emphasis added); *see also* A57, 5:24-29; A92, 5:5-10). Obviously larger microelectrodes, up to 1,000 μm in width, can provide better contact area than smaller microelectrodes, and provide more surface area for electrical signals to pass through.

Further, while the specification refers to dimensions of up to about 100 μm as being "preferred," the specification also explicitly recognizes the value of larger-dimensioned electrodes:

> The [electrode] arrays may be particularly useful as a component of an electrochemical sensor, where there is added value, benefit, or cost efficiency, to the use of a flexible substrate, or where there is ***value, benefit, or cost efficiency*** in having an interdigitated

> [electrode] array of ***dimensions relatively larger than the dimensions of interdigitated arrays conventionally disposed on non-flexible substrates.***

(A1767:26-31 (emphasis added); *see also* A57, 6:45-51; A92, 6:27-33).

The specification also expressly teaches that electrodes of increased width can be used in the invention:

> The use of an [electrode] array or sensor of the invention offers certain practical advantages. For instance, ***a flexible substrate can be used in combination with relatively larger-dimensioned electrodes, including electrode components of increased size (e.g., width)*** as well as increased spacing between them.

(A1779:11-14 (emphasis added); *see also* A62, 15:21-26; A96, 14:11-16).

The specification notes that larger-dimensioned micro-electrode arrays may be used, particularly where larger-sized microelectrodes provide cost and efficiency advantages:

> The use of ***larger-dimensioned micro-electrode arrays*** also allows the ***significant advantage of fabricating arrays and sensors using relatively less expensive and more efficient flex circuit photolithography processes***.

(A1762:27-29 (emphasis added); *see also* A55, 2:62-65; A90, 2:62-65).

In short, throughout the specifications, the applicant cites to the general understanding in the field, describes advantages of larger electrode configurations, and in no way limits the disclosure to the preferred embodiments of 100 μm or less.

2. The Originally Filed Claims in the Provisional Application Preclude Limiting the Issued Claims to the Electrode Widths of the Preferred Embodiments

The District Court failed to address other intrinsic evidence, including the claims as originally filed in the Provisional Application. *See Union Oil Co. v. Atlantic Richfield Co*., 208 F.3d 989, 998, n.4 (Fed. Cir. 2000) (original claims must be treated as part of the disclosure). Those claims confirm that the District Court erred in construing the "electrode" claim terms as being limited to microelectrodes having a width of up to approximately 100 μm.

Original claim 1 of the Provisional Application defined a microelectrode as "having a width greater than 15 μm" without any upper limit:

> 1. An array of micro-electrodes proximal to a flexible substrate, wherein the array comprises electrode elements having a width greater than 15 μm.

(A1793:3-4). By contrast, dependent claim 2 of the Provisional Application limited the microelectrodes of claim 1 to a subset of microelectrodes having a maximum width of "about 100 μm":

> 2. The array of claim 1 wherein electrode elements have a width in the range from greater than 15 μm to about 100 μm.

(A1793:6-7).

The District Court's construction of the term "electrode" effectively limits the claims of the patents-in-suit to the electrodes of claim 2 of the Provisional

Application. By definition, however, claim 1 of that application is broader than claim 2 and the District Court's construction.[9] *See* 35 U.S.C. § 112, fourth paragraph (dependent claims must impose further limitations on the subject matter of the independent claim referenced); *Baldwin Graphic Sys., Inc. v. Siebert, Inc.,* 512 F.3d 1338, 1345 (Fed. Cir. 2008) (independent claims "are naturally broader than their dependent counterparts.").

The District Court's construction not only improperly seeks to read back into the claims language the applicant dropped during prosecution, *see Laryngeal Mask Co. v. Ambu*, 618 F.3d 1367, 1372-73 (Fed. Cir. 2010) (rejecting construction that would add limitation previously dropped from claims),[10] but it also seeks to read back in a ***narrower*** limitation for "microelectrode" than was present in the original independent claim. The fact that the applicant felt it necessary to specify a subset of microelectrodes with a width of up to "about 100 μm" in dependent claim 2 makes it clear that the applicant did not believe the term "microelectrode," much less "electrode" itself, contained such a limitation. *Liebel-Flarsheim Co. v. Medrad, Inc.*, 358 F.3d

[9] Similarly, original claim 16 of the '891 application (which issued as the '146 patent) disclosed that the broad invention comprised electrodes having "a width of between about 15 μm and about 500 μm." (A1851:2-3).

[10] Citing *Kistler Instrumente AG v. United States*, 628 F.2d 1303, 1308 (Ct. Cl. 1980) (rejecting attempt to "read[] back into the claims limitations which were originally there and were removed during prosecution").

898, 910 (Fed. Cir. 2004); *Phillips*, 415 F.3d at 1314 (reference in claim to "steel baffles" made clear that baffles were not necessarily made of steel). The District Court's construction of "electrode" as narrower than the open-ended definition set forth in claim 1 of the Provisional Application is erroneous and impermissibly adds a limitation that may not be read into the claims.

3. The Prosecution History Explicitly Discusses "Microelectrodes" With Widths Over 100 µm (and Up to 1,000 µm)

The District Court's Post-Remand Opinion also fails to address Mr. Wilsey's March 1997 Excalibur Report, which was submitted with his declaration during the prosecution of both the patents-in-suit and therefore is part of the intrinsic evidence. That report describes the electrode configurations that were the focus of Mr. Wilsey's "Microelectrode Characterization" project as of March 1997. (A925). The microelectrodes described in the Excalibur Report had electrode widths of 1,000, 500, and 300 µm.

As shown at the bottom right of Fig. 2 below, taken from the Excalibur Report, the working electrode of the "box-hook microelectrode" configuration was 1,000 µm by 1,000 µm:

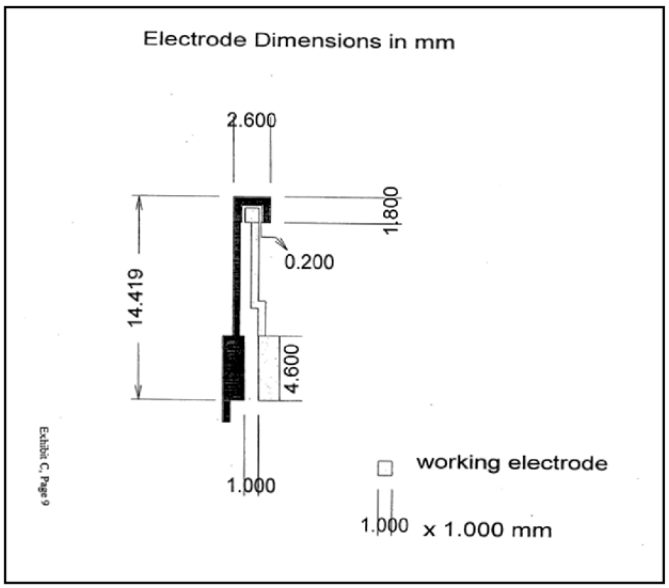

Fig. 2

(A926).

The Excalibur Report repeatedly refers to this 1,000 µm-wide box-hook configuration as a "microelectrode" (Fig. 3):

Electrochemical Area Determination via Chronocoulometry

Box-Hook Microelectrodes

Fig. 3: "Box-Hook Microelectrodes"

(A935; *see also* A941 (referring to "reused box-hook microelectrodes"); A948 (same); A954 (referring to "Microelectrode" with "Working electrode – 1 mm^2")).

The Excalibur Report also confirms that the 3-finger electrodes with widths of 500 μm are microelectrodes (Fig. 4):

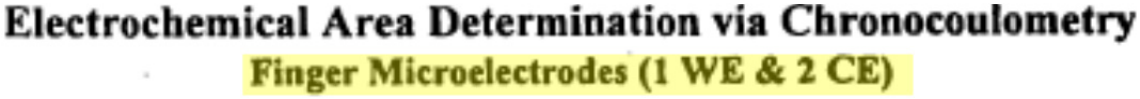

Fig. 4: "[3] Finger Microelectrodes"

(A936; *see also* A927 (showing each finger of this microelectrode configuration to be 500 μm wide)).

Moreover, the report confirms that the 5 finger electrodes with finger widths of 300 μm are microelectrodes (Fig. 5):

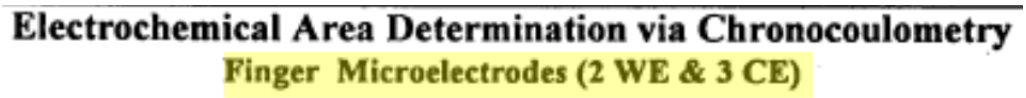

Fig. 5: "[5] Finger Microelectrodes"

(A937; *see also* A928 (showing four of the five fingers of this microelectrode configuration are 300 μm wide)).

The Excalibur Report is not some after-the-fact, litigation-induced document, but rather was created in 1997, during the development of Mr. Wilsey's invention. Moreover, this report was submitted to the Patent Office on March 5, 2003 in the prosecution of the '322/'147 application (*see* A6437-6438; A6487-6496) and on December 22, 2006 in the prosecution of the '891/'146

application (*see* A3715-3724; A3727-3729) – long before this dispute arose and under circumstances that had nothing to do with the width of "electrode." Notably, it was submitted in the '891/'146 application with the same Response in which, as discussed below, the claims were limited to blood and the first dependent claims with the 25-200 μm depth limitation were added. (A27260-27292). The inclusion of the Excalibur Report in connection with 2003 and 2006 inventor declaration submissions shows that the applicant continued to believe that the electrode terms encompassed the microelectrode configurations disclosed in that report, including microelectrodes up to approximately 1,000 μm in width.

This intrinsic evidence strongly supports the ordinary and customary meaning of "microelectrode" as electrodes up to at least 1,000 μm.

Further, one of the prior art references cited during prosecution of both patents-in-suit, U.S. Patent No. 5,250,439 ("Musho"), disclosed "microelectrodes" of up to "300 μm" in width. (A26375 at 30:20-21). Dr. Higson, one of Defendants-Appellees' experts, admitted that U.S. Patent No. 5,202,261, a divisional of Musho with this same disclosure, provides an "accurate depiction of what a microelectrode would be understood as, by a person of ordinary skill in the art." (A1319-1320, 194:15-199:4).

Accordingly, the prosecution history provides compelling evidence confirming that "microelectrodes" are not limited to 100 μm in width, and that electrodes having widths of 1,000, 500, and 300 μm are understood to be "microelectrodes." This intrinsic evidence fundamentally refutes the construction proposed by Defendants-Appellees and embraced by the District Court; under the Court's improper construction and as proposed by Defendants-Appellees, these electrodes from the prosecution histories would indisputably be classified as macroelectrodes, not microelectrodes.

4. The Purported "Disavowal" of Macroelectrodes Does Not Support a 100 μm Limitation

In its Pre-Remand Opinion, the District Court concluded that the patentee disavowed larger electrodes based on prosecution statements that purportedly distinguished certain prior art references (Morales, Mizutani I-III, and Rishpon). (A13-14). Even if the statements were to be viewed as a disavowal, the statements in no way disavowed widths up to 1,000 μm because ***none of the prior art references discussed in the response at issue had an electrode width that was less than 1,600 μm***.[11] Thus, the only widths that could have been disclaimed as macroelectrodes were widths of 1,600 μm or greater.

[11] Morales *et al.* describes an electrode in Fig. 1 that has a width of at least 4 mm (4,000 μm). (A1244). Mizutani I and II (both abstracts) do not set forth any

Although there is good reason to question whether these statements constitute a disavowal of macroelectrodes at all,[12] the statements distinguishing prior art references having electrode widths of 1,600 μm or more are entirely consistent with Roche's construction of the "electrode" terms as including microelectrodes having widths up to 1,000 μm.

C. The District Court Erred by Failing to Consider the Effect of Diffusion, Which Precludes a 100 μm Width Limitation on the "Electrode" Terms

In order to address the meaning of microelectrode, this Court instructed the parties to determine "what degree of non-planar diffusion justifies characterizing an electrode as a micro-electrode…." *Roche*, 452 Fed. Appx. at 995. In response, the District Court committed an error of law by dismissing diffusion altogether, stating "[t]here is some difficulty in converting the first

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particular electrode size. (A1250; A1251). Mizutani III uses an electrode that has a width of 1,600 μm ("gold disc electrode (diameter 1.6 mm...)"). (A1254). Rishpon (an abstract) also does not indicate any electrode dimensions. (A1259).

[12] First, the alleged disavowal was just one of several grounds for distinguishing these references within just a single Response. (A28032-28038 at A28036-28038). *See Ecolab, Inc. v. FMC Corp.,* 569 F.3d 1335, 1342 (Fed. Cir. 2009) ("Even if an isolated statement appears to disclaim subject matter, the prosecution history as a whole may demonstrate that the patentee committed no clear and unmistakable disclaimer."). Further, although the Examiner repeatedly cited numerous references disclosing electrodes with widths of ***4,000 μm*** (A260, 19:2-7; A1087; A1183, 19-21), ***3,000 μm*** (A1080; A1094), and ***1,500*** μm (A274, 3:58-59; A1087), the applicant never distinguished any of these references based on electrode size. (A1070-1071; A1024; A1298-1299; A1306-1307; A1933-1934).

characterization [where there is greater than 50% non-planar diffusion] into a size, as [the specification] gives no basis for doing so." (A1.16). In doing so, the District Court ignored both intrinsic and extrinsic evidence establishing that diffusion precludes limiting a microelectrode to a width of 100 μm.

First, the District Court adopted the "especial" situation, where "diffusion occurs predominantly (e.g., greater than 50%) according to a non-planar path":

> It is also understood that some electrode configurations can cause diffusion to take place by a mix of planar and non-planar paths, in which case the electrodes can be considered a micro-electrode array, ***especially*** if the diffusion occurs predominantly (e.g., greater than 50%) according to a non-planar path, or if the size of the electrodes is less than 100 μm, e.g., less than 50 μm.

(A56, 4:42-48; A91, 4:23-29) (emphasis added)). The District Court not only ignored the term "especially," but also ignored the primary phrase of the same sentence, which expressly states that ***any*** **"**mix of planar and non-planar" diffusion can be considered a microelectrode. The stated preference for predominant non-planar diffusion (along with a width of "less than 100 μm, e.g., less than 50 μm"), like other similar statements in the specification, is merely a non-limiting description of a preferred embodiment of the claimed invention.

Roche also proffered evidence adduced after the Pre-Remand *Markman* hearing from the testimony of Defendants-Appellees' experts. Nova's expert

Dr. Higson, for example, admitted that electrodes of up to 1,000 μm in width would exhibit some nonplanar diffusion:

> Q. Will a one-thousand-micron electrode width also experience non-planar diffusion [edge] effects?
>
> A. Yes.

(A27234, 209:6-9; *see also* A26242-26243, ¶9). LifeScan's expert, Dr. Williams, admitted that LifeScan's electrodes, which are more than 100 μm in width, would exhibit some non-planar diffusion. (A27226-27228, 71:19-81:3). Roche's expert, Dr. Weber, provided detailed evidence on diffusion patterns for electrodes of up to 1,000 μm in width, showing that they would exhibit substantial amounts of non-planar diffusion. (A26243-26245, ¶¶10-14; A26325-26328). For example, 1,000 μm microelectrodes would conservatively exhibit 52% non-planar diffusion with a 50 μm capillary height and 11.5% with a 100 μm capillary height. (A26245, ¶13; A26327-26328). In short, electrodes with widths up to at least 1,000 μm will indisputably display a mix of planar and nonplanar diffusion and, according to the express statement in the specification, "can be considered a micro-electrode." (A56, 4:43-45; A91, 4:24-26).

While it is clear from the admissions of Defendants-Appellees' experts and Dr. Weber's results that diffusion alone does not provide a clear demarcation of where a microelectrode ends and where a macroelectrode begins,

it is equally clear that electrodes of up to at least 1,000 μm in width will still exhibit some non-planar diffusion. A 100 μm width limitation on a microelectrode is also ***clearly inconsistent*** with the explicit statement in the specifications of the patents-in-suit that electrodes exhibiting a mix of planar and non-planar diffusion can be considered a microelectrode. (A56, 4:42-45; A91, 4:23-26).

As such, diffusion characteristics coupled with the specification's explicit statement that electrodes that exhibit "a mix of planar and non-planar [diffusion] can be considered a micro-electrode array" preclude a 100 μm width limitation on the "electrode" terms.

D. The District Court Erred in Disregarding Examples 3-5 of the '146 Patent, Which Disclose Microelectrodes Having Widths Up to 1,000 μm

The District Court also erred in rejecting Examples 3-5 of the '146 patent, which are concrete examples of the "larger-dimensioned electrodes" disclosed in the specification that employ electrodes of up to 1,000 μm in width.

1. The Intrinsic Evidence Identifies Examples 3-5 as "Microelectrodes"

The electrodes in Examples 3-5 are relevant, if not determinative, in construing "microelectrode." The electrode in Example 3 is Mr. Wilsey's 1 mm/1,000 μm-wide box-hook microelectrode. (A49, Fig. 9; A67, 26:25-27 and

33-34). The electrodes of Example 4 are Mr. Wilsey's 500 μm-wide 3-finger microelectrodes. (A68, 27:41-46). The electrodes of Example 5 are Mr. Wilsey's 300 μm-wide 5-finger microelectrodes. (A68, 28:21-27). These Examples from the '146 patent are part of the intrinsic record and therefore must be consulted to ascertain the meaning of the claims. *Union Oil Co.*, 208 F.3d at 998, n.4. As discussed above, all of these electrodes are clearly and fully described in the intrinsic prosecution history as "microelectrodes." (*See* A925-928; A935-937; A954).

These Examples cannot be rejected simply because they use glucose solutions instead of blood. Obviously, the solution applied to the microelectrodes has no effect on the width of the microelectrodes. A structural feature such as a "microelectrode" does not lose its designation simply because of the analyte applied to it. Such a construction would suggest that a "microelectrode" could mean one thing for tests using glucose solutions or serum (up to 1,000 μm), and another thing for tests in blood (only up to 100 μm). That distinction simply does not make sense for such a structural feature. By disregarding these Examples, the District Court failed to "consider the specification as a whole, and [to] read all portions of the written description, if possible, in a manner that renders the patent internally consistent" as mandated by this Court. *Pfizer*, 429 F.3d at 1373. Moreover, as the District Court noted,

"[t]he parties agree that 'electrode' should be construed the same way in the '146 and '147 patents." (A1.17).

On remand, the District Court rejected Examples 3-5 simply because they do not comport with the "microelectrode definition" it discerned from the "especially" characterizations that it identified from one sentence of the specifications. (A1.16-1.17). Yet that is exactly the type of construction that this Court rejected in *Pfizer*.

The District Court's construction is inconsistent with and directly contradicted by Examples 3-5 of the '146 patent. Thus, the claims must be construed so as to encompass the electrodes disclosed in these Examples, *i.e.*, up to approximately 1,000 μm.

2. The District Court Failed to Answer This Court's Fast Fill Question

This Court, with respect to Examples 3-5, noted that the linchpin of Defendants-Appellees' arguments for rejecting these Examples was their assertion that the specification of the '146 patent teaches that a capillary depth of less than 100 μm is not suitable for the "fast fill" of blood. This Court therefore asked the District Court on remand to determine exactly what "fast fill" means in the context of the patents-in-suit and whether it is relevant to the claims:

> There is also no dispute that the patents in suit generally aim to facilitate faster measurements (compared to the prior art) of glucose concentrations in small blood samples. But it is unclear whether the asserted claims are limited to the "fast fill" of blood. The parties have not sufficiently explained what "fast fill" means, and whether it is simply synonymous with the concept that the claimed invention is faster than the prior art, or whether the phrase has some other (perhaps specific) meaning.

Roche, 452 Fed. Appx. at 996. The short answer is that fill time, whether fast or not, is irrelevant to the claims as a matter of law. Yet the District Court on remand failed even to address this Court's question regarding the relevance of "fast fill" to the claims.

As this Court noted in its prior decision, the specification states that using capillaries having depths of 100 μm or more for blood ensures "fast fill" of blood over a broad range of hematocrits:

> Capillaries with depths of greater than or equal to 100 μm have been found to allow fast fill of blood with hematocrits from 20 to 70% to reliably flow into the chamber. Capillary depths of less than 100 microns to 25 microns can be used for other biological fluids such as serum, plasma, intersticial fluid, and the like.

(A64, 19:45-50; A98, 18:27-32). Neither "fast fill" nor any particular range of hematocrits is claimed in any of the claims of the patents-in-suit, however.

To the contrary, the claims of the patents-in-suit recite a test time that begins with "detecting the blood sample in the capillary chamber" and ends with a display of glucose concentration results "within 10 seconds after said detecting." (*See, e.g.,*

A102, claim 36 at 25:30, 33-35). But blood sample detection – which begins the claimed test time – occurs ***after*** the capillary chamber fills with blood. Put another way, the "fill time" (shown in red in Figure 6 at right) takes place at time X to time 0, ***before*** detection begins (at time=0) and thus before the claimed test time (shown in green) begins. Neither the fill time nor any "fast fill" feature is part of the claimed 10-second or less test time. The "fill time" – the time it takes for the blood sample to flow into and fill the capillary chamber to the point where it is detected – is simply irrelevant to the claims.

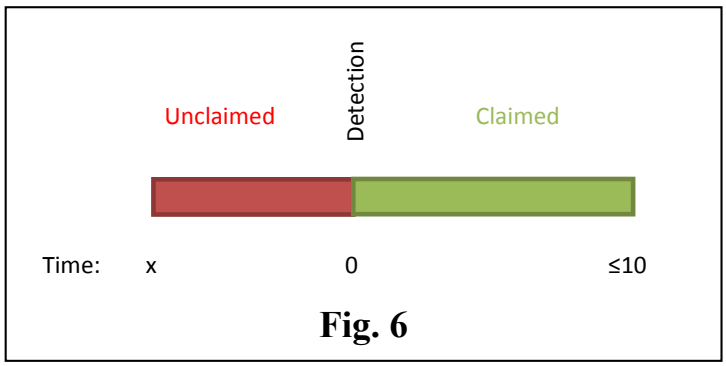

Fig. 6

Further, the range of hematocrit (20-70%) over which such "fast fill" with blood can be achieved is also irrelevant to the claims. (A64, 19:46-47; A98, 18: 28-29). None of the claims even mention hematocrit, much less claim operation over this broad range. The broad range is also far beyond normal hematocrit levels and well beyond any commercial requirements.[13]

[13] Hematocrit is the percentage of red blood cells in a blood sample. Extremely high amounts of hematocrit can impede blood flow. Normal hematocrit levels are 35 to 48%. (A27334-27335). LifeScan itself limits its Ultra product – one of the accused infringing products – to hematocrit ranges of 30-55%. (A27349, under "Limitations of Procedure").

As such, as a matter of law, the response to this Court's question as to whether the passage in the specification regarding "fast fill" is relevant to the claims is, simply, "no." Neither the fill time nor the broad hematocrit range addressed by that passage is claimed in any of the claims of the patents-in-suit. There is no basis for disregarding Examples 3-5 because their capillary depths may have impeded "fast fill." Rather, the "electrode" terms, including the term "microelectrode," must be construed as encompassing electrodes of up to 1,000 μm in width as taught in these Examples.

E. The District Court's "Enablement Lite" Analysis Based Solely on the Intrinsic Evidence Was Both Technically Flawed and Legally Improper

This Court noted that claim 48 of the '146 patent (like claim 53 of the '147 patent) recites using capillary depths of 25-200 μm for testing a blood sample, which indicates that the asserted claims cover capillary depth ranges found in Examples 3-5 of the '146 patent. *Roche*, 452 Fed. Appx. at 996. This Court also recognized that, to overcome this fact, Defendants-Appellees urged that claim 48 is not enabled, and that it was improperly "left-over" from the original claims. Thus, this Court asked the District Court on remand to answer several questions relating to the prosecution history:

> For example, the parties have not sufficiently answered the following questions: 1) when did Roche limit the asserted claims of the '146 patent from testing serum and blood to blood only, 2)

> was this change reflected in the dependent claims too? (and if not, should it have been?), 3) when did dependent claim 48 first appear in the '146 patent?

Id. at 996-97.

Further, this Court also asked the District Court on remand to consider "whether the referenced prosecution history—or any other evidence that the district court may admit into evidence in its discretion—can establish that examples 3, 4, and 5, as well as independent claim 48 in the '146 patent are not enabled and thus should not shed light on the scope of the asserted claims." *Id*. at 997, n. 6. The answers to these questions relating to Defendants-Appellants' enablement argument do not justify ignoring claim 48 (or Examples 3-5) in construing the claims.

1. Claim 48 of the '146 Patent (and Claim 53 of the '147 Patent) Was Not "Left Over"

The sequence of events in the prosecution history establishes that dependent claims containing the 25-200 μm depth limitation were not "left over," but were added by amendment at the same time the independent claims were limited to blood. In these claim amendments, the applicant explicitly cited the support from the specification for the claimed 25-200 μm range, and the Examiner thereafter allowed these claims after considering them on multiple

occasions. In short, the prosecution history provides no basis for finding lack of enablement of claim 48.

The claims originally filed in both applications were directed generally to "an analyte in a test sample," rather than specifying only blood. (A2101; A6474). On March 22, 2006, the applicant cancelled the original patent claims in the '891/'146 patent application and proposed new independent claims directed *inter alia* to testing "blood or serum" in a "capillary chamber having a depth of 25-200 μm." (A14247-14248). On June 5, 2006, the applicant made a similar change to the claims in the '322/'147 patent application. (A14299-14301).

On August 28, 2006, the Examiner issued non-final rejections in the '322/'147 application, including a rejection of the independent claims in that application for lack of written description under § 112, first paragraph. (A28204-28205; A28212-28214). The Examiner based this rejection on the "blood or serum" and "capillary chamber having a depth of 25-200 μm" limitations, using the same "fast fill" sentence discussed in the previous section, arguing that the specification did not "support[] using a capillary depth less than 100 μm for a blood sample for determining glucose concentration in the sample within about 10 seconds after said detecting" and "in fact teaches away from this

feature." (A28213). No corresponding § 112 rejection was made in the '891/'146 patent application.

On December 22, 2006, in both applications, the applicant filed further amendments that limited the claims to blood samples by deleting the reference to "serum" in the claims. (A27263; A27300). At the same time, the applicant removed the 25-200 μm depth limitation from the independent claims and placed that depth limitation into ***multiple*** separate, dependent claims in each of the applications (pending claims 71, 77 and 93 of the '891/'146 application and pending claims 55, 59 and 78 of the '322 /'147 application). (A27265-27269; A27303-27307). All of these new dependent claims – including the ones that ultimately issued in the '146 and '147 patents[14] – contained the ***exact same*** substantive text as issued claims 48 and 53: "The method of claim [X] in which said capillary chamber has a depth of 25-200 μm."

In these amendments in both applications, the applicant detailed the written description support for the new dependent claims. (A27275-27277; A27316-27317).[15] For the 25-200 μm depth limitation, the applicant cited

[14] Pending claim 71of the '891/'146 application issued as claim 16 of the '146 patent. (A6415). Pending claim 55 of the '322/'147 application issued as claim 19 of the '147 patent. (A10637).

[15] Appellants also note that, in each table containing references to the written description support, these 25-200 μm depth dependent claims were prominently

support in the specification: "Preferred dimensions of a capillary for what can be referred to as a 'low volume sensor configuration,' can be in the range of 0.025 mm [25 μm] to 0.2 mm [200 μm] (depth)…,". (A27275-27276, citing A60, 12:34-53; A27316, citing A95, 11:24-29). Neither the cited passage nor its surrounding text excludes the use of blood with such low volume configurations, or limits in any way what analytes can be tested with such configurations.

Based on these amendments and the cited support, the Examiner allowed the claims by Notices of Allowance dated February 6, 2007 and March 8, 2007 in the '891/'146 application, and February 6, 2007 in the '322/'147 application. (A4250-4260; A4276-4280; A28403-28412). Notably, the Examiner never interposed any written description rejection to the 25-200 μm depth limitation in the '891/'146 application, and apparently withdrew his rejection in the '322/'147 application based on the support cited in the December 22, 2006 amendment.

The District Court's opinion ignores that (1) the applicant added dependent 25-200 μm depth limitation claims in these December 22, 2006 amendments, and (2) the applicant provided written description support in those amendments for each of these claims. The District Court's failure to recognize

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positioned at the end, such that they would be impossible for the Examiner to "miss." (A27271-27277; A27310-27317).

that the applicant introduced these dependent claims with the 25-200 μm depth limitation in the December 22, 2006 amendments is important because those are the amendments in which the applicant explicitly cited the written description support for such claims, leading to their initial allowance. (A27275-27277; A27316-27317).

On March 23, 2007, the applicant filed petitions to withdraw both patents from issuance to submit additional prior art and to make amendments to the specification and the claims. (A4282-4299; A6307-6321; A6331-6399; A8498-8517; A10509-10513; A10519-10532; A10551-10560).

In April 2007, the applicant submitted further amendments in both applications regarding the 25-200 μm depth limitation claims:

- Canceling some of the claims in both applications (pending claims 77 and 93 of the ’891/’146 application and pending claims 59 and 78 of the ’322/’147 application), but leaving claim 71 of the ’891/’146 application and claim 55 of the ’322/’147 application (A14346-14351; A14361-14365); and
- Adding new 25-200 μm depth limitation claims: claim 135 of the ’891/’146 application (claim 48 of the ‘146 patent) and claim 127 of the ’322/’147 application (claim 53 of the ’147 patent). (A14353-14355; A14367-14369).

These dependent claims – all of which contain the identical depth limitation of 25-200 μm – were allowed once again by the Examiner in both applications on July 31, 2007. (A27507-27514; A27500-27505). No written description rejection was interposed by the Examiner during this continued examination to these 25-200 μm depth limitation dependent claims. No enablement rejection was ***ever*** interposed by the Examiner with respect to any of these claims.

The District Court's reliance on the initial written description rejection in one of these applications as evidence of lack of enablement (A1.18) – even assuming that its conflation of the written description and enablement issues is appropriate – disregards the give-and-take of patent prosecution. The District Court simply ignored that the Examiner did not maintain that rejection in the '322/'147 application but rather allowed the dependent claims in both applications once the applicant detailed adequate written description support in the specification.

The District Court's reliance on the initial written description rejection also disregards the presumption of validity and the added presumption that an Examiner who specifically addressed an issue did his job correctly. *See Am. Hoist & Derrick Co. v. Sowa & Sons, Inc.*, 725 F.2d 1350, 1359 (Fed. Cir. 1984). In allowing the claims that issued as dependent claim 48 of the '146 patent (and claim 53 of the '147 patent), the Examiner necessarily found that the

specification supported testing of blood samples within the full capillary depth range of at least 25-200 μm. *See Schindler Elevator Corp. v. Otis Elevator Co*., 593 F.3d 1275, 1284 (Fed. Cir. 2010) (actions "implicitly permitted" in dependent claim are "by extension" also permitted in the claim from which it depends). In short, the prosecution history does not, and cannot, support the District Court's "enablement lite" finding of lack of enablement for claim 48 of the '146 patent (or claim 53 of the '147 patent).

2. The District Court's Post-Remand "Enablement Lite" Analysis Failed to Address the Enablement Factors

The District Court's "enablement lite" analysis also ignored both this Court's enablement precedent and portions of the intrinsic evidence. As a result, this analysis was flawed as a matter of law. Although the District Court cited the enablement analysis required by this Court in *Cephalon, Inc. v. Watson Pharms., Inc.*, 707 F.3d 1330 (Fed. Cir. 2013), it failed to analyze any of the underlying factors required by this Court, applying instead an "enablement lite" analysis that apparently disregarded all of these factors. "Whether undue experimentation is required 'is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations.'" *Cephalon,* 707 F.3d at 1336 (citing *ALZA Corp. v. Andrx Pharms*., LLC, 603

F.3d 935, 940 (Fed. Cir. 2010) and *In re Wands*, 858 F.2d 731, 736–37 (Fed. Cir. 1988)).

Rather than weighing any of the requisite factual considerations, the District Court relied on its mistaken impression that the "fast fill" sentence in the specification precludes the use of blood in 25-200 μm deep capillary chambers and summarily concluded that "it would take undue experimentation to practice claim 48." (A1.19). Indeed, Defendants-Appellees failed to provide the District Court with any other evidence – including any expert opinions – in support of any of the enablement factors. (*See* A28420-28421, ¶13).

In stark contrast to the District Court's "enablement lite" analysis and Defendants-Appellees' failure to offer any other evidence supporting a lack of enablement, each of the cases cited by the District Court in its opinion addressed substantive expert testimony regarding application of the enablement factors. *See Forest Labs., Inc. v. Ivax Pharm., Inc*., 438 F. Supp. 2d 479, 487 n.3 (D. Del. 2006), *aff'd,* 501 F.3d 1263 (Fed. Cir. 2007) (applying the *Wands* factors and addressing expert evidence regarding enablement of prior art reference); *Robocast, Inc., v. Apple Inc.*, 39 F.Supp.3d 552, 565 (D. Del. 2014) (denying summary judgment of invalidity where opposing side's experts "have both opined that the Zellweger reference is not enabling. This makes the enablement of this reference a disputed factual issue.").

As discussed above in section III.D.2, however, "fast fill" over a broad range of hematocrits is irrelevant to the claims. The District Court's conclusory assertion that the specification "teaches that a capillary depth of less than 100 μm is not suitable for the capillary flow of blood," (A1.19), is wrong as a matter of law. Thus, the District Court's "enablement lite" analysis is also legal error.

The District Court also ignored Roche's evidence that Mr. Wilsey had contemporaneously and successfully conducted tests with blood using the same electrode and capillary configurations disclosed in Examples 4 and 5 – both with 62 μm capillary depths.[16] Although it invited supplementation with such evidence on remand (A1.15), the District Court failed to address such evidence establishing enablement of claim 48, presumably based on its unwarranted conclusion that Examples 4 and 5 (along with Example 3) pertained to unclaimed embodiments.

[16] (A26744:14-26754:15; A25314-25367; A26781) (Wilsey lab technician explaining the 3-finger and 5-finger microelectrode configurations in the Excalibur Report, including electrode dimensions and 62 μm capillary chamber depth); (A26773:4-26778:1; A26484-26741; A26781) (discussing 1997 tests in blood using 500 μm-wide 3-finger microelectrodes and 62 μm-depth capillary chamber of Example 4); (A26778:2-26779:13; A26786-27016; A27017) (discussing 1997 tests in blood using 300 μm-wide 5-finger microelectrodes and 62 μm-depth capillary chamber of Example 5); (A28420-28421, ¶13; A26475-26477) (Roche's expert discussing 1997 blood tests with these microelectrode configurations).

The District Court also improperly conflated enablement with written description. As this Court has made very clear, "[s]ince its inception, this court has consistently held that § 112, first paragraph, contains a written description requirement separate from enablement...." *Ariad Pharmaceuticals, Inc. v. Eli Lilly and Co*., 598 F.3d 1336, 1351 (Fed. Cir. 2010) (*en banc*). The Examiner never found that the dependent claims containing the blood and 25-200 μm depth limitations were not enabled, would require undue experimentation, or could not be practiced in blood using the structures disclosed in Examples 3-5. Moreover, the Examiner's allowance of dependent claims containing the 25-200 μm depth limitation indicate that the Examiner withdrew the initial written description rejection of such claims in the '322/'147 application.

Finally, the District Court's decision on remand failed to address the presumption of validity. Although the case law cited in the District Court's decision recognized a presumption that issued patents are enabled (*Forest Labs.,* 438 F.Supp.2d at 487; *Robocast*, 39 F.Supp.3d at 565), the District Court failed to address this presumption or the added deference due to an Examiner, who is presumed to have done his job correctly. *See Am. Hoist & Derrick Co.*, 725 F.2d at 1359. In essence, the District Court's decision does the opposite and presumes that the Examiner erred in granting the claims.

In summary, the intrinsic record provides no basis for finding that the depth limitation of 25-200 μm in claim 48 of the '146 patent (and claim 53 of the '147 patent) is not enabled, or for overcoming the presumption of validity and the deference due to an Examiner who considered the "fast fill" issue and withdrew his only claim objection based on that passage. The District Court erred by failing to consider in its "enablement lite" analysis Roche's evidence that the electrode structures of Examples 4 and 5 were successfully used to measure glucose concentrations in blood. The District Court also erred by failing to consider the electrode structures of Examples 3-5 in construing the electrode terms, each of which have a capillary depth (62 μm) that fall squarely within the 25-200 μm range of claims 48 and 53. (A67, 26:42-44; A68, 27:49-50 and 28:29-30).

Claim 48 thus provides another basis as to why the "electrode" terms must be construed to encompass microelectrodes having the widths specified in the Examples 3-5, *i.e.*, 1,000, 500, and 300 μm, respectively.

F. The District Court Improperly Ignored the Technical Textbook, Dictionary, and Encyclopedias Because of Its Misapprehension of the Intrinsic Evidence

Absent an express intent to define a term or a disclaimer, claim terms are to be given their ordinary and customary meaning as they would have been understood by a person of ordinary skill in the art. *Thorner,* 669 F.3d at 1365.

The Federal Circuit has "especially noted the help that technical dictionaries may provide to a court 'to better understand the underlying technology' and the way in which one of skill in the art might use the claim terms." *Phillips*, 415 F.3d at 1318 (citing *Vitronics Corp. v. Conceptronic, Inc.*, 90 F.3d 1576, 1584 n.6 (Fed. Cir. 1996) ("[T]echnical treatises and dictionaries ... are worthy of special note.")). "Dictionaries and technical treatises, which are extrinsic evidence, hold a 'special place' and may sometimes be considered along with the intrinsic evidence when determining the ordinary meaning of claim terms." *Bell Atlantic Network Services, Inc. v. Covad Communications Group, Inc.*, 262 F.3d 1258, 1267 (Fed. Cir. 2001). Although the District Court noted in its decision the various cautionary statements this Court has made, such as "the use of [a] dictionary may extend beyond what should properly be afforded by the inventor's patent" and that there is "no guarantee that a term is used in the same way in a treatise as it would be by the patentee," the District Court provided no analysis as to why such cautions should apply here. (A1.20). Here, the extrinsic materials are all remarkably consistent, both between themselves and with the intrinsic evidence that the District Court rejected in its analysis.

The scientific textbook, *Electrochemical Methods, Fundamentals and Applications*, by Bard & Faulkner (2001 2nd ed.), is the standard reference text in the field of the invention as confirmed by the testimony of Defendants-

Appellants' experts. (A1310, 16:19-17:2 (Dr. Williams: "Bard & Faulkner is the standard text in the field...."); A1317, 188:10-189:22 (Dr. Higson testified he used Bard & Faulkner and probably cited it in his own work)). Bard & Faulkner defines microelectrodes as having widths of up to 1 mm, or 1,000 μm ("micrometer" dimensions and "a 1-mm diameter microelectrode") (A1047; A1050). It also indicates that smaller electrodes on the order of the District Court's construction – smaller than 25 μm radius, or 50 μm width – are a subset of microelectrodes, known as "ultramicroelectrodes." (A1049). In contrast, Bard & Faulkner defines "normal" electrodes, or macroelectrodes, in terms of "meters, centimeters, or millimeters." (A1049).

This conventional definition of "microelectrodes" has not changed substantially over time. The founding fathers of electrochemistry, Laitinen and Kolthoff, published on microelectrodes having a width (diameter) of 500 μm in 1939. (A1324). Nearly 70 years later, the 2008 *Electrochemical Dictionary* continued to define microelectrodes as having widths up to 1 mm (1,000 μm):

> Microelectrode – Electrode with a characteristic dimension ranging from 25 μm up to 1 mm.

(A1055; *see also* A1056-1057 (defining smaller electrodes, with a width of 25 μm or less, as "ultramicroelectrodes")). Defendants-Appellees' experts admitted

that this technical dictionary is also a standard reference book in the field of electrochemistry. (A1310 at 16:19-17:2; A1317 at 188:10-189:22).

Similarly, the 1994 Edition of the *Kirk-Othmer Encyclopedia of Chemical Technology* defines a microelectrode as up to 1 mm in diameter: "Small, referring to the diameter of the electrode, is about a millimeter for microelectrodes, or perhaps only a few micrometers for ultramicroelectrodes...." (A27527). The 2005 Edition applies the exact same definition. (A27639).

In short, each of these technical references is fully consistent with the ordinary and customary meaning of "microelectrode," i.e., measured in micrometers (microns) as opposed to millimeters. These technical references also support – and certainly do not "trump" – the intrinsic evidence, including the repeated references in the specification teaching the use of "larger-dimensioned electrodes"; the repeated references in the specification to electrodes of 100 μm in width or less as merely preferred embodiments; the 1,000, 500, and 300 μm-wide microelectrodes in Examples 35 of the '146 patent; the Excalibur Report in the prosecution history of both patents describing the 1,000, 500, and 300 μm-wide electrodes as microelectrodes; and the Musho '439 reference, cited during prosecution of both patents-in-suit, describing 300 μm-wide electrodes as microelectrodes. As such, these references confirm what the intrinsic evidence already makes apparent: that the correct construction of

the "electrode" terms should include microelectrodes having a width of up to approximately 1,000 μm.

The District Court rejected these references without analysis, solely because they would "trump" the District Court's definition. It is the District Court's definition, however, that is flawed, because it relied on just a portion of a single sentence in the Detailed Description describing a preferred embodiment of the invention, while failing to consider all of the intrinsic evidence as required by *Pfizer,* or the ordinary meaning of the term. The indisputable definitions in these technical references confirm the intrinsic evidence as a whole and the ordinary meaning establishing that "microelectrode" includes electrodes up to approximately 1,000 μm in width. As such, this Court should construe the "electrode" terms to mean "microelectrodes having a width of 15 μm up to approximately 1,000 μm."

IV. The District Court Erred in Applying the Third Circuit Standard For Reconsideration, Particularly in Light of This Court's Rolling Claim Construction Precedent and Defendants–Appellees' Waiver

A. The District Court Improperly Considered its Claim Construction Under the Third Circuit Reconsideration Standard Rather than the Rolling Claim Construction Standard Applied by This Court

In addressing whether to reach the merits of claim construction post-remand, the District Court mistakenly applied the Third Circuit standard for

reconsideration articulated in *Max's Seafood Cafe v. Quinteros*, 176 F.3d 669, 677 (3d Cir. 1999). As this Court has repeatedly recognized, however, *Markman* rulings are interlocutory orders and, as such, "district courts may engage in a rolling claim construction, in which the court revisits and alters its interpretation of the claim terms as its understanding of the technology evolves." *Pressure Products Med. Supplies, Inc. v. Greatbatch Ltd.*, 599 F.3d 1308, 1316 (Fed. Cir. 2010); *see also Conoco, Inc. v. Energy and Env't Int'l, L.C.*, 460 F.3d 1349, 1359 (Fed. Cir. 2006); *Jack Guttman, Inc. v. Kopykake Enters., Inc.*, 302 F.3d 1352, 1361 (Fed. Cir. 2002); *In re Papst Licensing Digital Camera Patent Litigation*, 778 F.3d 1255, 1261 (Fed. Cir. 2015) ("it is worth reiterating that a district court may (and sometimes must) revisit, alter, or supplement its claim constructions (subject to controlling appellate mandates) to the extent necessary to ensure that final constructions serve their purpose of genuinely clarifying the scope of claims for the finder of fact"); *Lexington Luminance LLC v. Amazon.com Inc.*, No. 2014-1384, 2015 WL 524270, n. 5 (Fed. Cir. Feb. 9, 2015) ("on remand, the district court may supplement its claim constructions consistent with the controlling appellate mandates as the case moves forward"); *Wright Asphalt Products Co., LLC v. Pelican Refining Company, LLC*, CA H-09-1145, 2012 WL 1936416, *12 (S.D. Tex. May 29, 2012) ("The Federal Circuit, however, appears to suggest that these demanding standards for

reconsideration do not apply when a party is seeking reconsideration of a claim-construction opinion. Instead, a district court is free to revise its claim construction if its 'evolved' understanding of the technology makes it appropriate.").

Because the Pre-Remand *Markman* briefing and hearing occurred before any invalidity discovery and before any expert discovery (A2047-2051; A2040-2041), a rolling claim construction standard is particularly appropriate here. Moreover in the context of this case, the District Court specifically invited "the parties to supplement the record as they saw fit" in the Post-Remand *Markman* proceedings. (A1.15). As such, the District Court's application of the Third Circuit reconsideration standard was improper, and the District Court should have applied a rolling claim construction standard consistent with this Court's decisions.

B. The District Court Erred In Its Application of the Third Circuit Standard

Even under the Third Circuit reconsideration standard, reconsideration was appropriate based on "the availability of new evidence that was not available when the court granted the motion for summary judgment" and "the need to correct a clear error of law or fact or to prevent manifest injustice." *Max's Seafood*, 176 F.3d at 677. In this case, the District Court's failure to

address the intrinsic evidence that both (a) establishes that a "microelectrode" extends up to at least 1,000 μm and (b) is inconsistent with the District Court's 100 μm limitation constitutes a clear error in claim construction. Allowing such a construction to stand would give rise to a manifest injustice. In addition, evidence that was not available at the time of the Pre-Remand *Markman* hearing – including the admissions of Defendants-Appellees' experts that electrodes of more than 100 μm in width will exhibit a mix of planar and non-planar diffusion and the additional evidence regarding diffusion submitted after remand at the District Court's invitation – confirms the legal error in the pre-remand (and post-remand) claim construction. As such, even under the Third Circuit reconsideration standard, the construction that imposes a width limitation of approximately 100 μm should be reversed. The District Court's interlocutory order proffering an improper claim construction was, by definition, "contrary to law." *See Castro*, 671 F.3d at 365 (quotation marks omitted).

C. Defendants-Appellees Waived Any Argument that the Reconsideration Evidence – Including the Supplemental Evidence Proffered by Both Sides Post-Remand – Should Not Be Addressed on the Merits

Finally, Defendants-Appellees have waived any objection to reconsideration both pre- and post-remand. The issue of the appropriateness of reconsideration and whether Defendants-Appellees preserved their objection

came from the District Court *sua sponte* during the Post-Remand *Markman* hearing. The District Court then requested supplemental briefing on the issue. (A29055:12-29058:13). It was in response to this supplemental briefing that the District Court found that Defendants-Appellees' "response was underwhelming" and that it did "not see any evidence that Defendants put the Federal Circuit on notice that Defendants were arguing that the Federal Circuit should affirm the claim construction on the basis of a waiver argument." (A1.14). Nor did Defendants-Appellees make any such preservation of their objection at oral argument before this Court. (*See* A1.14).

Indeed, in the briefing relating to claim construction that followed the Pre-Remand Opinion, Defendants-Appellees relied upon substantial evidence that was proffered only on reconsideration.[17] They did so without attempting to distinguish that evidence and without reserving any procedural objection. As this Court found in the previous appeal, "Nova and Lifescan do not dispute on appeal, however, that Roche's argument should be addressed on the merits." *Roche*, 452 Fed. Appx. at 994. (*See also* A1.13).

[17] New evidence cited by Defendants-Appellees after the original Pre-Remand *Markman* includes A21151-31; A21121-37; A21138-40; A21141-42; A21143-45; A21154-56 (on pre-remand reconsideration) and A1974-78; A21143-45; A21143-45; A11445-46; A13896-99; A16303-04; A1317-18; A1318-20; A21116-7; A21122-30; A21118-9; A21138-40, A21141-42; A20074-82; A21132-37; A21127-28 (in the previous appeal).

Nor did Defendants-Appellees raise a procedural issue post-remand. To the contrary, in response to the District Court's invitation to "the parties to supplement the record as they saw fit" (A1.15), they supplemented the record further, citing to additional extrinsic references and submitting a declaration of Professor Higson, one of their experts.[18] Finally, Defendants-Appellees' Joint Answering Brief On Remand Regarding "Electrode" Construction is wholly devoid of any discussion of the standards for reconsideration or those standards not being met. (*See, e.g.,* A27761-27762 and A27769-27771). "An issue is waived unless a party raises it in its opening brief, and for those purposes a passing reference to an issue will not suffice to bring that issue before this court." *Skretvedt v. E.I. DuPont De Nemours*, 372 F.3d 193, 202-03 (3d Cir. 2004) (citation omitted).

CONCLUSION

For the foregoing reasons, this Court should construe the "electrode" terms as "microelectrodes having a width of 15 μm up to approximately 1,000 μm." Moreover, because the District Court's order granting Defendants-Appellees' motions for summary judgment was predicated on that erroneous

[18] Defendants-Appellees cited at least a dozen new exhibits and deposition transcripts in the Post-Remand *Markman,* separate and apart from the prosecution histories. (A27859-61 *et seq.*).

claim construction, the Court should reverse the District Court's claim construction of the "electrode" terms; vacate the District Court's Post-Remand *Markman* Order (A1.5), Order on summary judgment (A1.3-1.4) and Final Judgment (A1.1-1.2); and remand this case to the District Court for further proceedings consistent with this Court's decision.

STATEMENT OF ORAL ARGUMENT

Oral argument is respectfully requested.

Respectfully submitted,

Dated: April 21, 2015

By: /s/ Grantland G. Drutchas

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ADDENDUM

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IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

ROCHE DIAGNOSTICS OPERATIONS, INC., and CORANGE INTERNATIONAL LIMITED, Plaintiffs, v. ABBOTT DIABETES CARE, INCORPORATED, and ABBOTT DIABETES CARE SALES CORPORATION, et al., Defendants.	C.A. No. 07-753 RGA

~~[PROPOSED]~~ FINAL JUDGMENT

For the reasons set forth in the Order granting Roche Diagnostics Operations, Inc.'s and Corange International Ltd.'s, (collectively "Roche's") and LifeScan Incorporated's ("LifeScan's") and Nova Biomedical Corporation's ("Nova's") (collectively, "Defendants") Stipulation and Joint Motion for Entry of Final Judgment of Non-Infringement ("Joint Motion") (D.I. 1029) following the Court's December 5, 2014 memorandum and order construing the term "electrode" as "microelectrode hav[ing] a width of 15 µm up to approximately 100 µm" (D.I. 1025, 1026);

IT IS HEREBY ORDERED AND ADJUGED that under the Court's construction of "electrode," LifeScan's and Nova's accused blood glucose monitoring systems[1] do not infringe the asserted claims of Roche's U.S. Patent Nos. 7,276,146 and 7,276, 147 because they do not have microelectrodes having widths of 15 µm up to approximately 100µm, and that Judgment be

[1] LifeScan's OneTouch® Ultra®, OneTouch® Ultra2®, OneTouch® UltraSmart®, OneTouch® UltraMini®, OneTouch® UltraLink™, and OneTouch® Select™ blood glucose monitoring systems and the Nova Max™, Nova Max Link™, BD Logic®, BD Paradigm Link®, BD Latitude, GlucoFix Mio, and GlucoMen LX blood glucose monitoring product lines in conjunction with compatible test strips.

and is hereby entered in favor of Defendants, LifeScan and Nova, on non-infringement of Roche's U.S. Patents 7,276,146 and 7,276,147.

So Ordered this 16th day of January, 2015.

Richard G. Andrews, U.S. District Court Judge
for the District of Delaware

1178293

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

ROCHE DIAGNOSTICS OPERATIONS, INC., and CORANGE INTERNATIONAL LIMITED, Plaintiffs, v. ABBOTT DIABETES CARE, INCORPORATED, and ABBOTT DIABETES CARE SALES CORPORATION, et al., Defendants.	C.A. No. 07-753 RGA

[~~PROPOSED~~] ORDER

Before this Court is Roche Diagnostics Operations, Inc.'s and Corange International Ltd.'s, (collectively "Roche's") and LifeScan Incorporated's ("LifeScan's") and Nova Biomedical Corporation's ("Nova's") (collectively, "Defendants"; collectively with Roche, the "Parties") Stipulation and Joint Motion for Entry of Final Judgment of Non-Infringement ("Joint Motion") (D.I. 1029) following the Court's December 5, 2014 memorandum and order construing the term "electrode" as "microelectrode hav[ing] a width of 15 μm up to approximately 100 μm" (D.I. 1025, 1026);

WHEREAS, for the reasons set forth in the Joint Motion, the Parties stipulate that under the Court's construction of "electrode," LifeScan's and Nova's accused blood glucose monitoring devices do not infringe the asserted claims of Roche's U.S. Patent Nos. 7,276,146 and 7,276, 147 (the "patents-in-suit") because they do not have microelectrodes having widths of up to approximately 100μm;

WHEREAS, the above stipulation of non-infringement of the asserted claims of the patents-in-suit as construed by the Court leaves no further issues on the merits before the Court at this time;

WHEREAS, for the reasons set forth in the Joint Motion, the Parties further stipulate that all motions for attorneys' fees and/or to declare the case exceptional and accompanying briefs in support shall be deferred until the exhaustion of all appeals consistent with D.I. 982, 941 and 927;

IT IS HEREBY ORDERED that the Parties' Stipulation and Joint Motion for Entry of Final Judgment of Non-Infringement is GRANTED and a judgment shall be entered.

IT IS FURTHER HEREBY ORDERED that, notwithstanding the timeframes provided by the Local Rules of the District of Delaware, and consistent with D.I. 982, 941, and 927, any motion for attorneys' fees and/or to declare the case exceptional and accompanying briefs in support thereof is hereby deferred until the latter of (i) thirty (30) days after the Federal Circuit issues its final mandate following exhaustion of all appeals, or (ii) thirty (30) days after the time to file any further appeals is exhausted.

So Ordered this 16th day of January, 2015.

Richard G. Andrews, U.S. District Court Judge
for the District of Delaware

1178302

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

Roche Diagnostics Operations, Inc., and **Corange International Ltd.,** Plaintiffs, v. **Abbott Diabetes Care, Inc., Abbott Diabetes Care Sales Corp., Bayer Healthcare, LLC, Diagnostic Devices, Inc., Lifescan, Inc.,** and **Nova Biomedical Corp.,** Defendants.	Civil Action No. 07-753-RGA

ORDER

This 5th day of December 2014, for the reasons stated in the accompanying memorandum opinion, IT IS HEREBY ORDERED that:

The term "electrode" is construed as "microelectrode have a width of 15 μm up to approximately 100 μm."

Richard G. Andrews
United States District Judge

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

Roche Diagnostics Operations, Inc., and **Corange International Ltd.,** Plaintiffs, v. **Abbott Diabetes Care, Inc., Abbott Diabetes Care Sales Corp., Bayer Healthcare, LLC, Diagnostic Devices, Inc., Lifescan, Inc.,** and **Nova Biomedical Corp.,** Defendants.	Civil Action No. 07-753-RGA

MEMORANDUM OPINION

Philip A. Rovner, Esq., Potter Anderson & Corroon LLP, Wilmington, DE; Grantland G. Drutchas, Esq. (argued); attorneys for Plaintiffs Roche Diagnostics Operations, Inc. and Corange International, Ltd.

Mary W. Bourke, Esq., Womble Carlyle Sandridge & Rice, LLP, Wilmington, DE; William C. Rooklidge, Esq. (argued); Jones Day, Irvine, CA, attorneys for Defendant LifeScan, Inc.

Rodger D. Smith, II, Esq., Morris Nichols Arsht & Tunnell LLP, Wilmington, DE; Bradford J. Badke, Esq. (argued), Ropes & Gray, LLP, New York, NY, attorneys for Defendant Nova Biomedical Corp.

December 5, 2014

ANDREWS, U.S. District Judge:

Pending before the Court, on remand from the United States Court of Appeals for the Federal Circuit, is consideration of the construction of the term "electrode."

On September 15, 2009, the Court construed the claimed "electrode" as "microelectrode having a width of 15 μm up to approximately 100 μm." *Roche Diagnostics Operations, Inc. v. Abbott Diabetes Care*, 667 F.Supp.2d 429, 435-36 (D.Del. 2009) (the "*Markman Decision*"); (D.I. 563 & 564). Two weeks later, Roche moved for reconsideration, positing a different claim construction theory than the one it had previously advanced. (D.I. 636). On January 21, 2010, at the second pretrial conference, the Court denied the motion for reconsideration. (D.I. 852 ¶ 4). On July 27, 2010, the Court granted summary judgment of non-infringement for Defendants LifeScan and Nova. (D.I. 850 at 2 ¶¶ 2 & 3). Roche appealed the Court's summary judgment order as being predicated, in part, on the erroneous claim construction of "electrode." (D.I. 852 ¶ 2). On January 25, 2012, the Federal Circuit vacated the judgment of non-infringement and remanded to this Court to consider the parties' arguments that pertain to the scope of the term "electrode." *Roche Diagnostics Operations, Inc. v. LifeScan Inc.*, 452 F. App'x 989, 998 (Fed. Cir. 2012) (the "*CAFC Decision*"). This Court duly heard argument on the "electrode" construction. For the reasons set forth below, the Court holds that the proper construction of "electrode" is "microelectrode having a width of 15 μm up to approximately 100 μm."

I. PROCEDURAL ISSUES

Roche filed this lawsuit in 2007, alleging patent infringement, against various defendants including Lifescan and Nova. The case followed the normal course of hotly-contested patent litigation. In due course, the Court held a Markman hearing. In preparation for the hearing, the parties submitted briefing. One of the terms in dispute was "electrode."

Roche did not originally specify any proposed construction for "electrode." (D.I. 357 at

6) (listing three terms for construction, not including "electrode"). Defendants proposed that the claimed "electrode" should be construed as a "microelectrode having a width of 15 to 100 μm." (D.I. 359 at 19) (*E.g.*, "a working electrode" is "a working microelectrode having a width of 15 to 100 μm"). Roche responded to Defendants' proposed claim construction, arguing that the claims were not limited to microelectrodes (D.I. 380 at 12-14) and were not limited to "having a width smaller than 100 μm." (*Id.* at 14-23); *CAFC Decision*, 452 F. App'x at 992 ("[A]t the claim construction stage, Roche argued . . . that the term 'electrode' in the asserted patent claims includes both 'micro' and 'macro' electrodes. Roche asserted that micro-electrodes are up to approximately 100 μm wide.") .

The Court rejected Roche's proposed construction and concluded that "the claims should . . . be limited to microelectrodes." *Markman Decision*, 667 F. Supp.2d at 435; *see also id.* ("[T]he written description repeatedly confirms that the invention, and hence the claims, are directed to methods utilizing microelectrodes."); *CAFC Decision*, 452 F. App'x at 994-95 ("Roche agrees . . . that the claims do not cover all electrodes of all widths. [Roche] now concedes that the term 'electrode' only covers micro-electrodes, not macro-electrodes."). The Court also addressed the parties' arguments concerning the preferred dimensions of microelectrodes. Not surprisingly, as all parties agreed that the upper limit of a microelectrode was "approximately 100 μm," *see id.* at 992 ("Roche asserted that microelectrodes are up to approximately 100 μm, the Court concluded that the claimed 'microelectrodes' had a width of 15 μm up to approximately 100 μm. The Court also noted that the specification "does not describe the upper limit of the range as a strict cutoff" and, therefore, the construction "illuminates the

size of the microelectrode to one of skill in the art without improperly excluding microelectrodes that are *slightly* larger than the preferred dimensions." *Markman Decision*, 667 F. Supp. 2d at 436.

Roche moved for reconsideration of the claim construction, not seeking to reargue that the asserted claims read only on microelectrodes (D.I. 636, p.1), but arguing that microelectrodes may have widths up to 1000 μm. In support of the motion, Roche identified five bases – one being "[n]ewly obtained extrinsic evidence" and the other four being various "[e]rror[s] of apprehension ([l]aw])." (*Id.*, pp. 2-3). At a pretrial hearing on January 14, 2010, the Court stated, "I've read the briefing. I'm not convinced that I made a mistake or that I didn't consider all the arguments. So what I'm saying is that I would maintain the claim construction, but I'm going to give you an opportunity to put something in place to tell me that I shouldn't." (D.I. 858 at 47:7-13). After the hearing, the Court entered an order inviting an opposition brief to the Court's tentative denial of Roche's motion for reconsideration of the claim construction order. (O.O., Jan. 14, 2010).

Roche submitted the requested briefing on January 19, 2010. (D.I. 774). The briefing dealt solely with a prosecution history argument based on *Martek Biosciences Corp. v. Nutrinova, Inc.*, 579 F.3d 1363 (Fed. Cir. 2009), which was decided less than two weeks before the Court's claim construction ruling. (D.I. 774 at 4). Roche contended that *Martek* required the Court to consider prosecution history as a whole, rather than in a piecemeal fashion, and set forth the rule that preferred examples do not limit the claims to those preferred examples. (*Id.* at 5). *Martek* was not cited in any of Roche's previous briefing regarding reconsideration of the claim

construction ruling. Thus, this briefing was essentially a second motion for reconsideration. The Court held a second pretrial hearing on January 21, 2010, during which the Court stated, "I've looked at the paper on reconsideration. It's a great point for the Federal Circuit, and I actually think you might have a point. But it will be interesting to see what they say. So we will be moving ahead with that Rule 54 judgment." (D.I. 859 at 5:24-6:6). The Court then denied the motion for reconsideration and entered summary judgment of non-infringement based on the "electrode" construction. (D.I. 850). Roche appealed. (D.I. 852).

The unique procedural posture presented an interesting set of circumstances on appeal. The Federal Circuit stated, "The district court did not address whether reconsideration was procedurally appropriate, and, if so, whether Roche's argument has merit." *CAFC Decision*, 452 F. App'x at 994. It is not clear to me which reconsideration argument (or arguments) the Federal Circuit was addressing when it made this statement. Under Third Circuit law, reconsideration is appropriate if there is: "(1) an intervening change in the controlling law; (2) the availability of new evidence that was not available when the court granted the motion for summary judgment; or (3) the need to correct a clear error of law or fact or to prevent manifest injustice." *Max's Seafood Café v. Quinteros*, 176 F.3d 669, 677 (3d Cir. 1999) (citing *North River Ins. Co. v. CIGNA Reins. Co.*, 52 F.3d 1994, 1218 (3d Cir. 1995)). The District Court considered Roche's initial argument for reconsideration and initially determined that it did not meet the standards for reconsideration. (D.I. 858 at 47:7-13) ("I'm not convinced that I made a mistake or that I didn't consider all the arguments."). The Court then allowed Roche to submit more briefing, which Roche did, making one argument that was not in the original motion for reconsideration. Roche

used the opportunity to present a second motion for reconsideration (even if not denominated as such). It seems clear to me that it was this subsequent argument that the District Court described as making "a great point." I say that both because the District Court had already rejected the arguments raised in the first motion for reconsideration, and because the language "a great point" seems more appropriate for a one point argument than a five point argument. The District Court followed up by entering an order denying without discussion the motion for reconsideration. The second argument for reconsideration appears to have been a non-starter in the Federal Circuit, as *Martek* and its related argument are not mentioned in the *CAFC Decision*.

The Federal Circuit thus appears to have conducted its review based on the content of the first motion for reconsideration combined with the Court's comment on the second argument for reconsideration. *CAFC Decision*, 452 F. App'x at 993 ("Roche moved the district court for reconsideration, positing a different claim construction theory. This time, Roche . . . argued that micro-electrodes may indeed be up to 1,000 μm wide. Roche also submitted new extrinsic evidence to support its motion for reconsideration. At the hearing for the motion for reconsideration, the district court remarked that Roche's new claim construction argument raised 'a great point.'").

After remand, at the September 5th hearing, I asked the parties to address (1) whether Roche's motion for reconsideration was procedurally appropriate, and (2) if so, whether Defendants waived any procedural objections to Roche's new claim construction argument by not addressing them on appeal to the Federal Circuit. Defendants argue that Roche did not appeal the Court's denial of reconsideration, *see* D.I. 1008, pp. 5-6, and therefore should be

foreclosed from now rearguing the claim construction. Defendants do acknowledge that Roche listed the denials of reconsideration in the notice of appeal, *see* D.I. 852, at ¶¶(3) & (4), but state that since Roche did not brief those issues on appeal, Defendants were under no obligation to raise the issue, and have waived nothing. (D.I. 1008 at pp. 11-13).

Motions to reconsider are disfavored. *See* D. Del. LR 7.1.5(a) ("Motions for reargument shall be sparingly granted."). The motion for reconsideration (D.I. 636) was based on "new extrinsic evidence" and four legal arguments. The "new extrinsic evidence" was mostly new only in the sense that it had not been presented by Roche before. For example, Roche relied primarily upon a 1993 U.S. patent (*id.*, p. 5), a 2001 standard text and a 2008 technical dictionary (*id.*, pp. 6-7), and a 1939 journal article. (*Id.*, p.8). It also found support in the 2008 reexamination proceedings. (*Id.*) It also claimed support from snippets of Defendants' experts' depositions, although the experts disagreed with Roche's position. The experts' depositions carry no independent weight, and everything else was available to Roche before the claim construction hearing. Roche offers no explanation why it should be getting a second bite at claim construction based on its "new extrinsic evidence." In my opinion, the "new extrinsic evidence" was not new and therefore was not a proper ground for reconsideration.

Roche raised four legal arguments. Roche couched them as errors of apprehension. Yet I do not think that is a fair characterization. The District Court understood Roche's argument. Indeed, it understood the importance of "electrode" among the mass of terms the parties wanted construed, *see Markman Decision*, 667 F. Supp. 2d at 434, and understood the parties' different positions. In my opinion, it is fair to say that the error of apprehension was Roche's, not the

Court's, as Roche argued a position in the claim construction hearing that it has subsequently partially abandoned. It is true that it raises new lines of analysis in support of its legal conclusion, but it is no different than moving for reconsideration of a point based on non-controlling authority one could have raised in the first instance. "Rolling claim construction" is not justified by a party's considered claim construction choices that it later regrets. There was no intervening change in the controlling law, and there was no clear error of law. I think to some extent the Federal Circuit's decision shows that there was no clear error of law. The Court's extensive and detailed analysis shows a host of unanswered questions. Thus, in my opinion, the Motion for Reconsideration (D.I. 636) was properly denied on procedural grounds. *See, e.g., Golden Bridge Tech., Inc. v. Apple Inc.*, 758 F.3d 1362, 1369 (Fed. Cir. 2014) ("An argument made for the first time in a motion for reconsideration comes too late and is ordinarily deemed waived."). Thus, on that basis alone, I adopt the District Court's original claim construction. I think such a decision is particularly warranted here where Roche is not only raising an argument it did not make, but an argument that is contrary to the argument it did make.

I do not, however, decide this issue in a vacuum. I am cognizant that a district court must respect any controlling decision of a court of appeals. The Federal Circuit noted, "Nova and Lifescan do not dispute on appeal, however, that Roche's argument should be addressed on the merits." *CAFC Decision*, 452 F. App'x at 994. That statement could be interpreted as either (1) any procedural issues about the propriety of the motion to reconsider are waived, or (2) despite any procedural issues about the propriety of the motion to reconsider, the parties have argued the merits without any objection. On the one hand, the Federal Circuit's statement sounds like a

conclusion that any procedural objections were waived. On the other hand, Defendants have represented that they did not waive the arguments in their briefing. (*See* D.I. 1021, at 38 ("[W]e were very clear in our briefs."). When I pressed them on the point, the response was underwhelming. Defendants cite to places in their briefs before the Federal Circuit where they "preserved and did not waive their arguments about the reconsideration standard not being met," (D.I. 1023 at 2), but the provided citations do not show any argument at all about reconsideration being procedurally improper. (*See* D.I. 1009-3 at 13, 15, 35; D.I. 1009-4 at 18-19, 21). I do not see any evidence that Defendants put the Federal Circuit on notice that Defendants were arguing that the Federal Circuit should affirm the claim construction on the basis of a waiver argument. As a result, the Federal Circuit cited no Third Circuit cases, or indeed any cases at all, in connection with either how the district court treats a motion to reconsider, or how a court of appeals treats the denial of a motion to reconsider. Exactly what the Federal Circuit decided, if anything, about the continuing ability of this Court to address procedural failures in the way the claim construction issue was handled is a question that I have pondered without coming to a conclusion in which I am confident during the unforgivably long period of time that this decision has been under advisement.

The Federal Circuit recognized that the motion for reconsideration raised the issue "whether reconsideration was procedurally appropriate," *CAFC Decision*, 452 F. App'x at 994, and that the District Court did not address it. I think that, having found the District Court's handling of the reconsideration motion was insufficient for lack of explanation, it is unlikely the Federal Circuit then decided essentially the same issue with no discussion at all. Thus, on

balance, I think the Federal Circuit did not actually decide the motion to reconsider issue. Having stated my view that the "procedural appropriateness" issue is dispositive, I nevertheless think the better course is to proceed from this point as if it were not dispositive.

II. THE MERITS

While the Federal Circuit considered reaching Roche's arguments, the Court noted that the record was not fully developed. *CAFC Decision*, 452 F. App'x at 996. The District Court had a record at the Markman hearing that contained everything the parties wanted to submit. Roche submitted with its first motion for reconsideration three volumes including 28 exhibits. (D.I. 637-39). If the record after a Markman hearing, a first motion for reconsideration, and a second argument for reconsideration was insufficient to support a basis for concluding the argument in Roche's favor, why should Roche get a fourth shot at making its case? The Federal Circuit "le[ft] it to the discretion of the district court whether and to what extent each party should be allowed to supplement the record with additional briefing and evidence . . . ." *CAFC Decision*, 452 F. App'x at 997. Since it appeared that I was going to need to reconsider the claim construction in light of the issues identified by the Federal Circuit, it seemed to me to be in the interest of justice to allow the parties to supplement the record as they saw fit. They did so, raising a discovery dispute along the way. (D.I. 993-94).

The Federal Circuit noted four issues for this Court to address. First, the Federal Circuit asked this Court to address "what degree of non-planar diffusion justifies characterizing an electrode as a [microelectrode]." *CAFC Decision*, 452 F. App'x at 995. Second, the Federal Circuit asked this Court to address whether examples 3, 4, and 5 of the '146 patent are

unclaimed embodiments. *Id.* at 996. Third, the Federal Circuit asked this Court to address whether "claim 48" is enabled. *Id.* at 996-97. Fourth, the Federal Circuit asked this Court to address whether it should consider new extrinsic evidence presented by Roche in support of its new claim construction. *Id.* at 997.

With respect to the Federal Circuit's first question concerning non-planar diffusion, in my opinion, the specification answers this question. Specifically, the specification states:

> It is also understood that some electrode configurations can cause diffusion to take place by a mix of planar and non-planar paths, in which case the electrodes can be considered a [microelectrode] array, especially if the diffusion occurs predominantly (e.g., greater than 50%) according to a non-planar path, or if the size of the electrodes is less than 100 μm, e.g., less than 50 μm.

'147 patent at col.4 ll.23-29. The specification, thus, seems to indicate that an electrode might be characterized as a microelectrode in one of two situations: (1) where there is greater than 50% non-planar diffusion, or (2) where the electrode has a width less than 100 μm. There is some difficulty in converting the first characterization into a size, as it gives no basis for doing so. As Roche admits, "diffusion simply depends on far too many variables to be limited to any particular size electrode." (D.I. 988, p.17). It would also make no sense to describe a microelectrode as being either (1) less than 1000 μm wide, or (2) less than 100 μm wide. The second characterization supports the Court's construction. What is clear to me is that neither characterization supports Roche's 1000 μm microelectrode construction.

Second, the Federal Circuit asked this Court to address whether examples 3, 4, and 5 of the '146 patent are unclaimed embodiments. The Court's original claim construction considered these examples and concluded that "such examples cannot pertain to claimed embodiments

because, although the claims are limited to blood samples, the examples include a capillary depth insufficient for the flow of blood." *Markman Decision*, 667 F.Supp.2d at 435. The parties agree that "electrode" should be construed the same way in the '146 and '147 patents. The '146 and '147 patents share a common specification, but examples 3, 4, and 5 only appear in the '146 patent. These examples must be read in light of the microelectrode definition in the common specification. *Sinorgchem Co., v. ITC*, 511 F.3d 1132, 1138 (Fed. Cir. 2007) ("Where, as here, multiple embodiments are disclosed, we have previously interpreted claims to exclude embodiments where those embodiments are inconsistent with the unambiguous language in the patent's specification or prosecution history."). Therefore, consistent with the Court's original claim construction, the Court concludes that examples 3, 4, and 5 are unclaimed embodiments.

The Court asked whether claim 48 of the '146 patent is enabled. Enablement is a question of law based on underlying factual findings. The issue is whether, at the time of filing the patent application, one skilled in the art, having read the specification, could practice the invention without "undue experimentation." *Cephalon, Inc. v. Watson Pharms., Inc.*, 707 F.3d 1330, 1336 (Fed. Cir. 2013). When enablement is asserted as an invalidity counterclaim, the parties have a right to a jury trial on it, and the underlying facts have to be proved by clear and convincing evidence. I do not imagine, however, that the Federal Circuit was asking me to have a trial on claim 48 before construing the patent. Instead, I think what the Federal Circuit had in mind was more the "enablement lite" sort of analysis that sometimes arises in connection with prior art references. *See, e.g., Robocast, Inc. v. Apple Inc.,* 2014 WL 1622002, *10 n.5 (D. Del. Apr. 22, 2014); *Forest Labs,., Inc. v. Ivax Pharm., Inc.*, 438 F. Supp. 2d 479, 487 n.3 (D. Del.

2006), *aff'd*, 501 F.3d 1263 (Fed. Cir. 2007).

Claim 48 of the '146 patent recites capillary depths of 25-200 μm and depends from claim 31. The prosecution history reveals that claim 48 is not enabled. Roche originally filed claims in both the '146 and '147 patent applications that were broadly directed to "an analyte in a test sample," rather than specifying only blood. (*See* D.I. 987, Ex. 24 at 45; *id.*, Ex. 20 at 36). On June 5, 2006, Roche cancelled the original '147 patent claims and proposed new claims directed to testing "blood or serum" in a "capillary chamber having a depth of 25-200 μm." (D.I. 987, Ex. 21 at 3). On August 28, 2006, the Examiner rejected those claims for lack of written description given the specification's capillary depth requirement, noting that the specification did not "support using a capillary depth less than 100 μm for a blood sample for determining glucose concentration in the sample within about 10 seconds after said detecting." (*Id.*, Ex. 22 at 9). The Examiner further stated that "[t]he specification in fact teaches away from this feature." (*Id.*).

On December 22, 2006, Roche narrowed both the '146 and '147 patent claims to test strips "including a capillary chamber having a depth suitable for capillary flow of blood." (D.I. 986, Ex. 20 p.4 [*see* D.I. 991-17 at 5]; D.I. 987, Ex. 21 p.4 [*see* D.I. 992-1 at 5]). In its remarks, Roche stated that the claims had been limited to testing blood using strips having "a depth suitable for capillary flow of blood." (D.I. 986, Ex. 20 pp. 25-26 [*see* D.I. 991-17 at 26-27]; D.I. 987, Ex. 21 p.28 [*see* D.I. 992-1 at 29]). However, on April 20, 2007, Roche added dependent claim 48 into the '146 application, which used the same "25-200 μm" capillary depth language that the Examiner had previously rejected for lack of written description. (D.I. 1002-6 at 100 ¶

135; *id.* at 115 ¶ 127). The claims must be read in light of their specification. The specification teaches that a capillary depth of less than 100 μm is not suitable for the capillary flow of blood. Thus, in order for a person of ordinary skill in the art to practice the invention of claim 48, that person would have to figure out how to obtain suitable capillary flow of blood in a capillary chamber which the specification teaches is not suitable. There is no information in the patent that would assist in doing this, and thus I conclude that it would take undue experimentation to practice claim 48. (It also appears, as the Examiner concluded when focusing on the issue, that claim 48 would be invalid for lack of written description.). The Court, thus, concludes that claim 48 is not enabled, and its existence does not alter the Court's construction of microelectrode.

Finally, with respect to the new extrinsic evidence presented by Roche in support of its new claim construction, the Court has considered it but does not find this evidence persuasive. A court may consider extrinsic evidence, including expert and inventor testimony, dictionaries and learned treatises, in order to assist it in understanding the underlying technology, the meaning of terms to one skilled in the art and how the invention works. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1318-19 (Fed. Cir. 2005) (en banc); *see also Markman v. Westview Instruments, Inc.*, 52 F.3d 967, 979-80 (Fed. Cir. 1995)(en banc), *aff'd*, 517 U.S. 370 (1996). Extrinsic evidence, however, is considered less reliable and less useful in claim construction than the patent and its prosecution history. *Phillips*, 415 F.3d at 1318-19 (discussing "flaws" inherent in extrinsic evidence and noting that extrinsic evidence "is unlikely to result in a reliable interpretation of a patent claim scope unless considered in the context of intrinsic evidence"). The Federal Circuit

specifically has cautioned against reliance on dictionaries because "the use of [a] dictionary may extend beyond what should properly be afforded by the inventor's patent." *Id.* at 1322. Indeed, the Federal Circuit has noted that "even technical dictionaries or treatises . . . may suffer from some of these deficiencies" and that there is "no guarantee that a term is used in the same way in a treatise as it would be by the patentee." *Id.* The Court concludes that Roche's dictionary references do not trump the intrinsic evidence already considered by this Court.

Accordingly, the Court affirms its earlier construction of the term "electrode" as a "microelectrode having a width of 15 μm up to approximately 100 μm."

III. CONCLUSION

For the reasons set forth in this Memorandum Opinion, the Court affirms its earlier claim construction of the term "electrode."

An Order consistent with this Opinion will be entered.

US007276146B2

(12) **United States Patent**
Wilsey

(10) **Patent No.: US 7,276,146 B2**
(45) **Date of Patent: *Oct. 2, 2007**

(54) **ELECTRODES, METHODS, APPARATUSES COMPRISING MICRO-ELECTRODE ARRAYS**

(75) Inventor: **Christopher D. Wilsey**, Carmel, IN (US)

(73) Assignees: **Roche Diagnostics Operations, Inc.**, Indianapolis, IN (US); **Corange International Limited**, Hamilton (BM)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 245 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/264,891**

(22) Filed: **Oct. 4, 2002**

(65) **Prior Publication Data**

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US 2007/0170055 A2 Jul. 26, 2007

Related U.S. Application Data

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(51) **Int. Cl.**
G01N 27/327 (2006.01)

(52) **U.S. Cl.** **205/792**; 205/777.5; 204/403.04

(58) **Field of Classification Search** 204/403.01–403.15; 205/777.5, 778, 792
See application file for complete search history.

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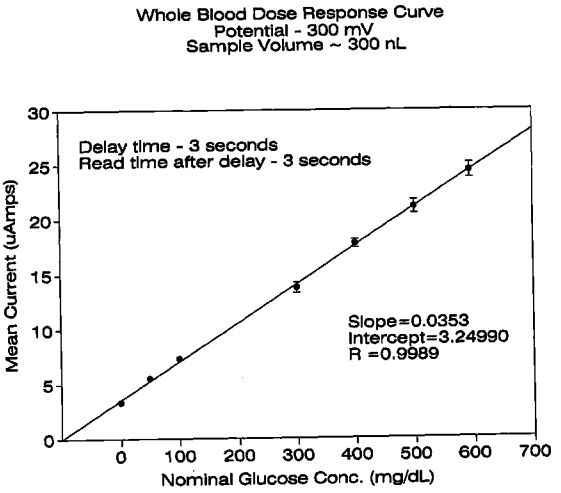
(74) *Attorney, Agent, or Firm*—Woodard, Emhardt, Moriarty, McNett & Henry LLP

(57) **ABSTRACT**

Described are micro-arrays of electrodes disposed proximal to a flexible substrate, electronic components and sensors comprising such arrays, and methods of use for such arrays.

62 Claims, 13 Drawing Sheets



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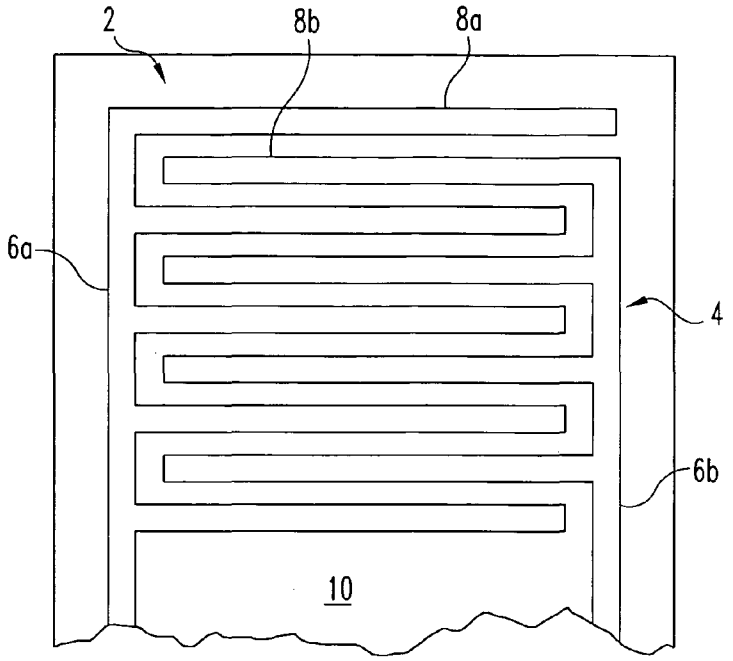


Fig. 1

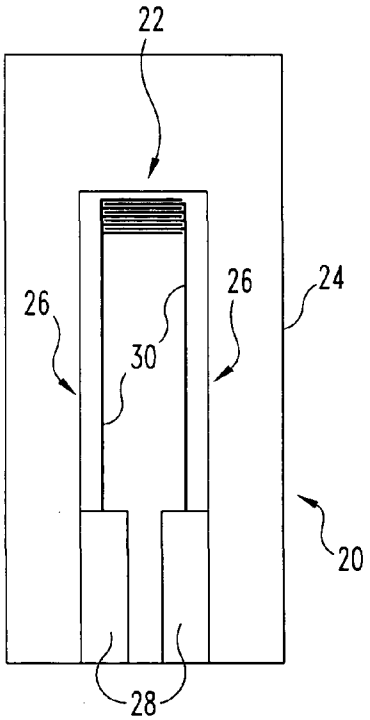


Fig. 2

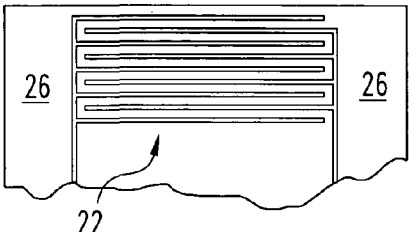


Fig. 2a

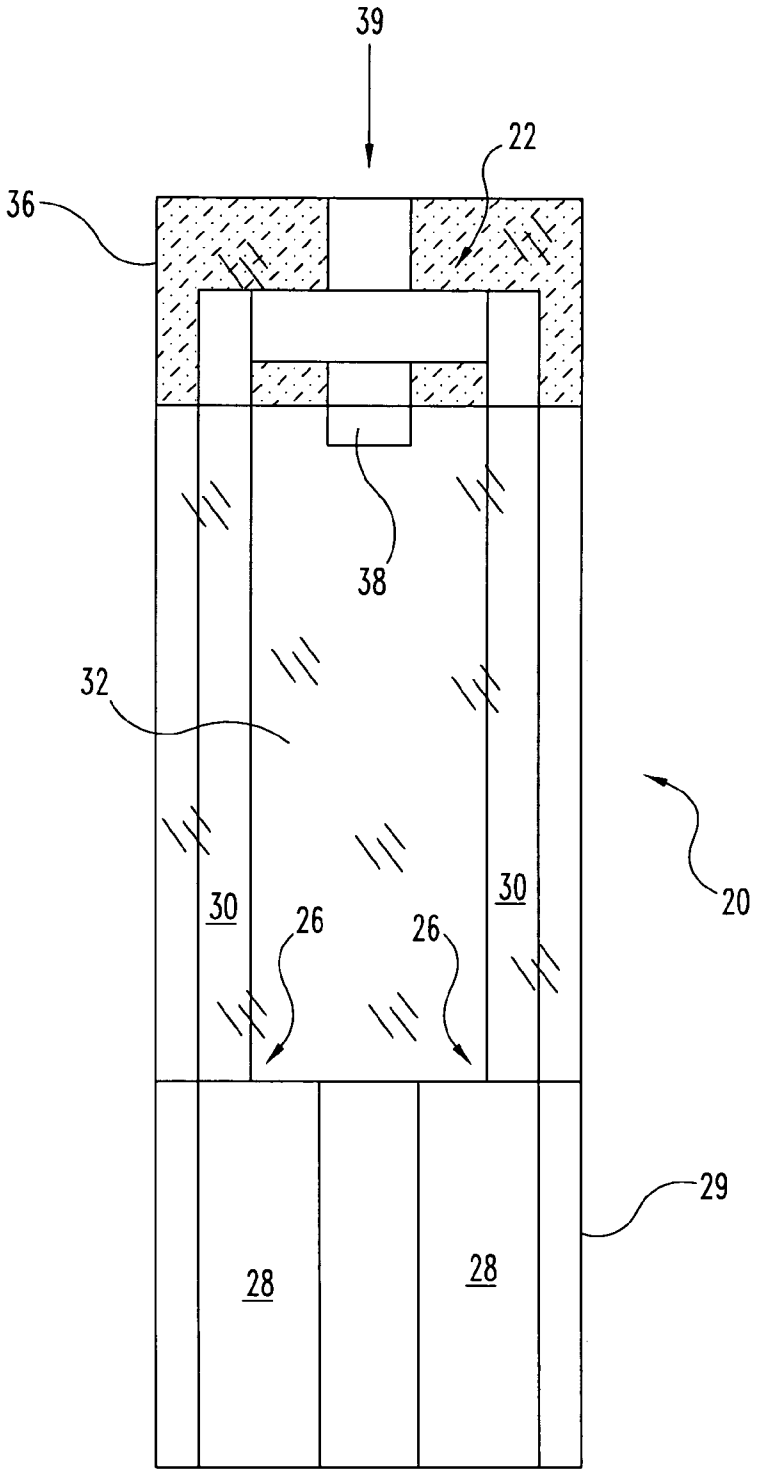


Fig. 3

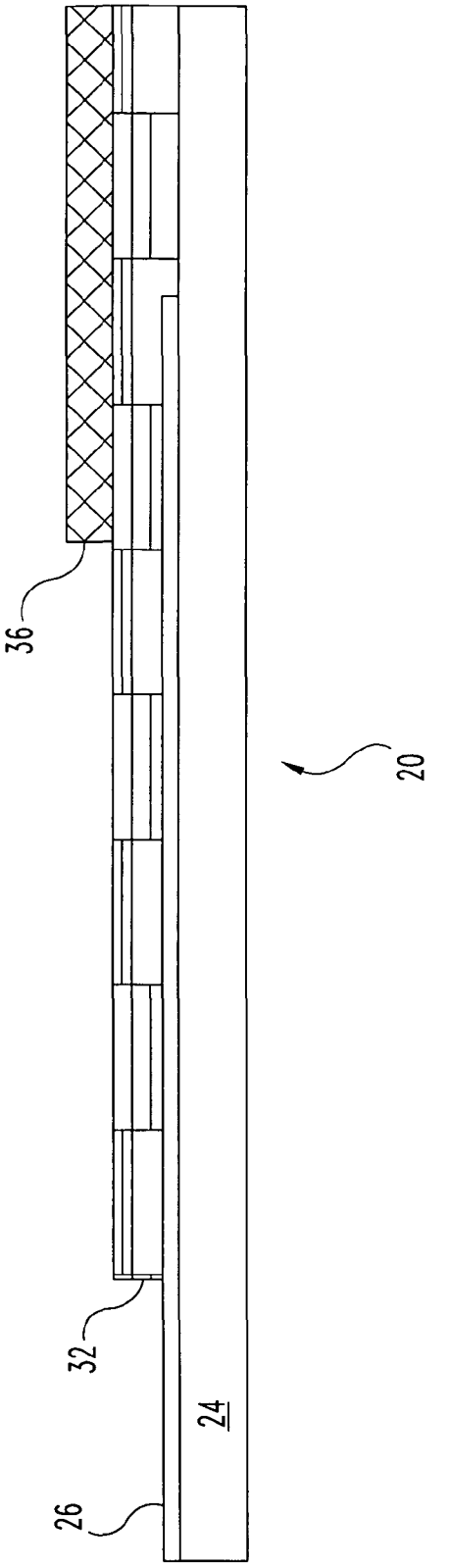


Fig. 4

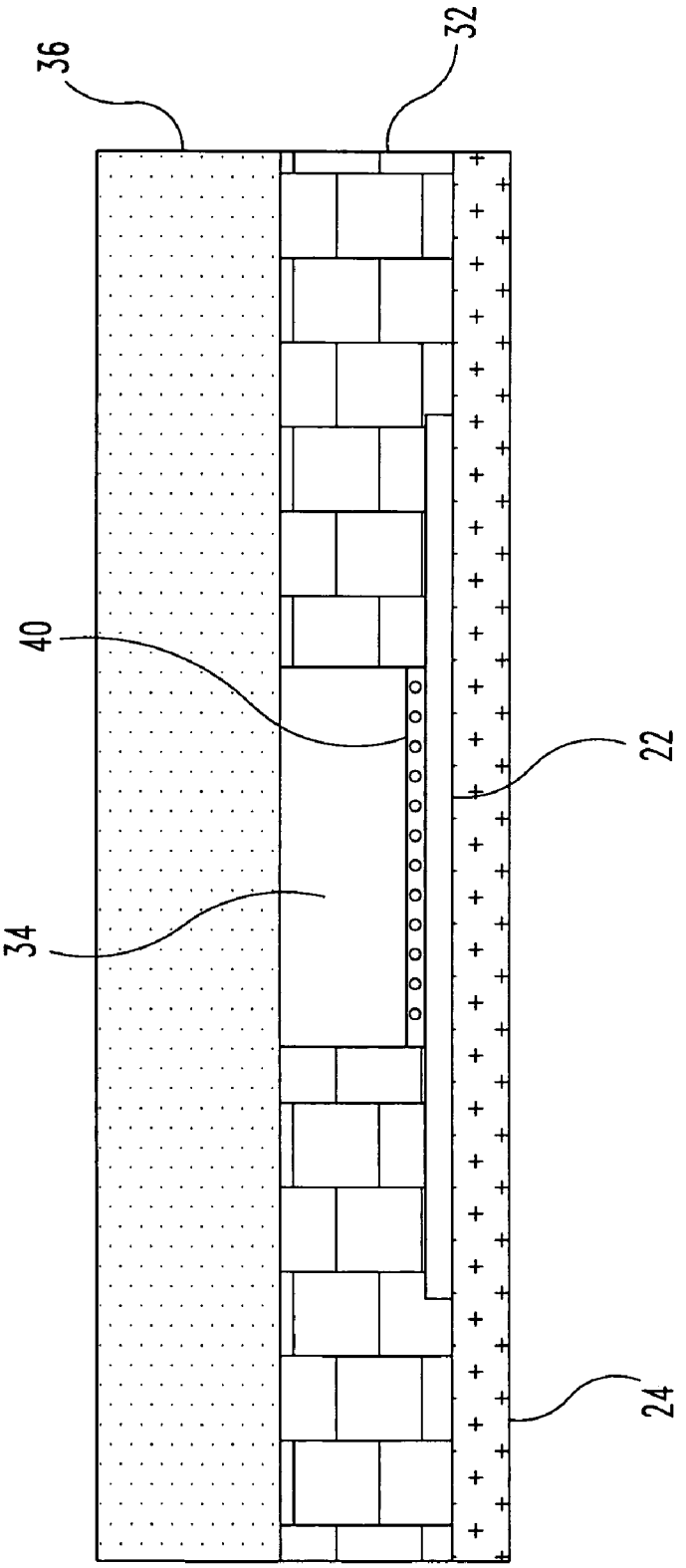


Fig. 5

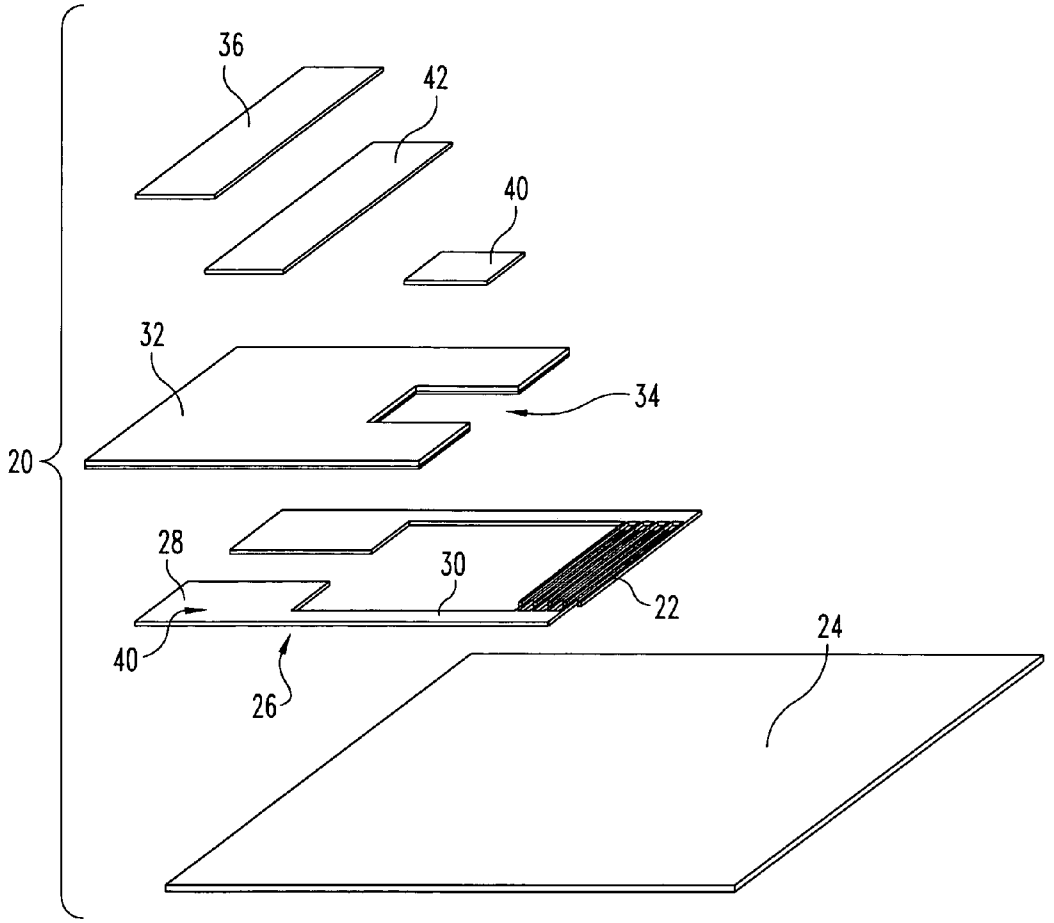


Fig. 6

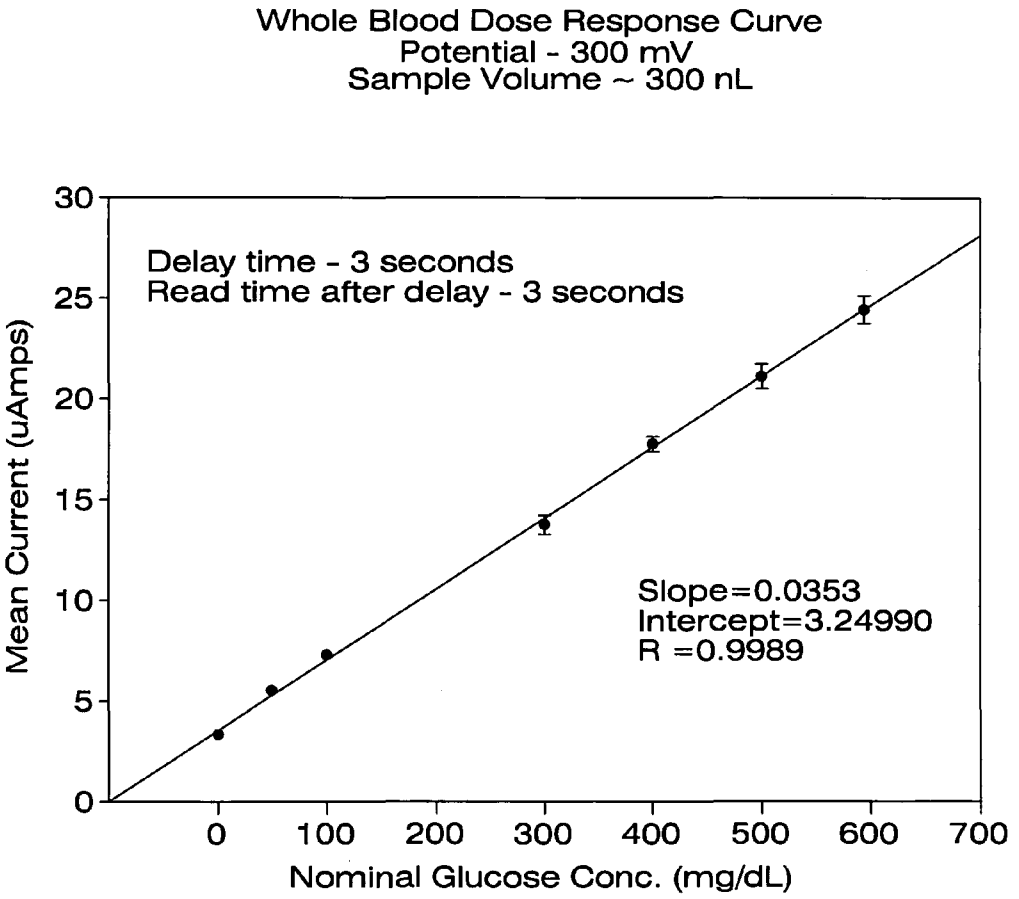

Whole Blood Dose Response Curve
Potential - 300 mV
Sample Volume ~ 300 nL
Delay time - 3 seconds
Read time after delay - 3 seconds
Slope=0.0353
Intercept=3.24990
R =0.9989
Mean Current (uAmps)
Nominal Glucose Conc. (mg/dL)
0
5
10
15
20
25
30
0
100
200
300
400
500
600
700

Fig. 7

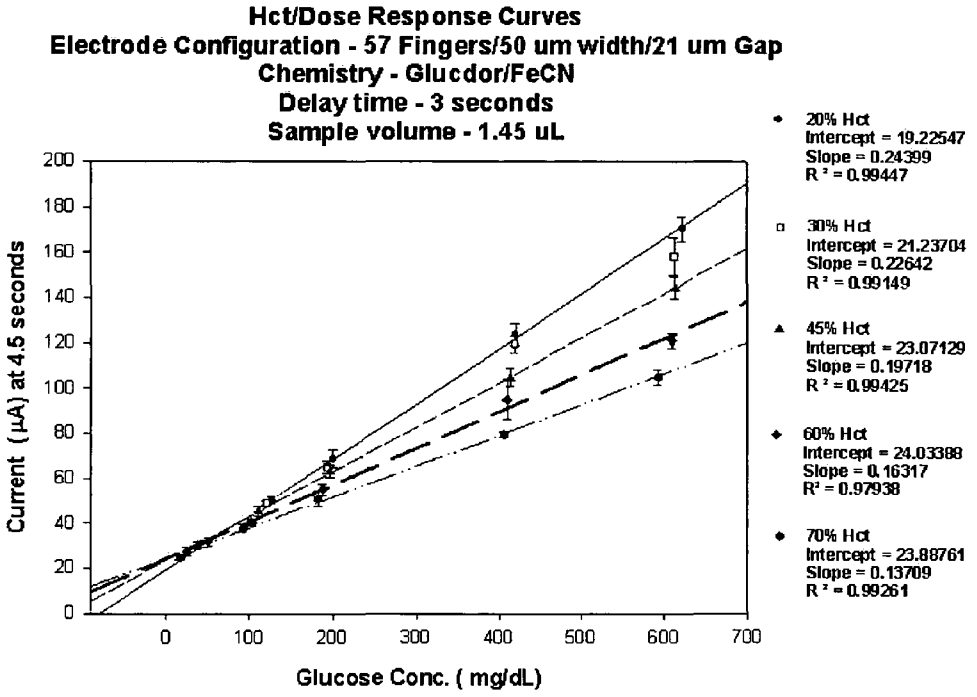

Hct/Dose Response Curves
Electrode Configuration - 57 Fingers/50 um width/21 um Gap
Chemistry - Glucdor/FeCN
Delay time - 3 seconds
Sample volume - 1.45 uL
Current (µA) at 4.5 seconds
Glucose Conc. (mg/dL)
0
20
40
60
80
100
120
140
160
180
200
0
100
200
300
400
500
600
700
20% Hct
Intercept = 19.22547
Slope = 0.24399
R ² = 0.99447
30% Hct
Intercept = 21.23704
Slope = 0.22642
R ² = 0.99149
45% Hct
Intercept = 23.07129
Slope = 0.19718
R ² = 0.99425
60% Hct
Intercept = 24.03388
Slope = 0.16317
R² = 0.97938
70% Hct
Intercept = 23.88761
Slope = 0.13709
R ² = 0.99261

Fig. 8

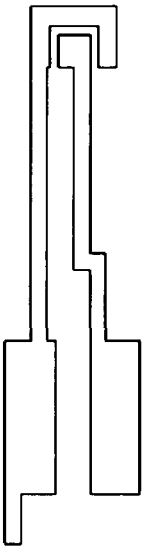


Fig. 9

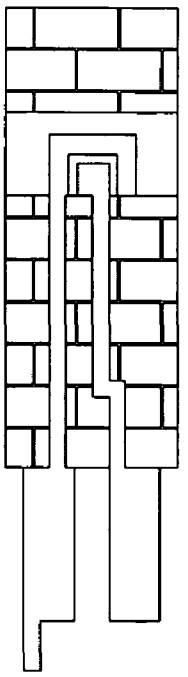


Fig. 9A

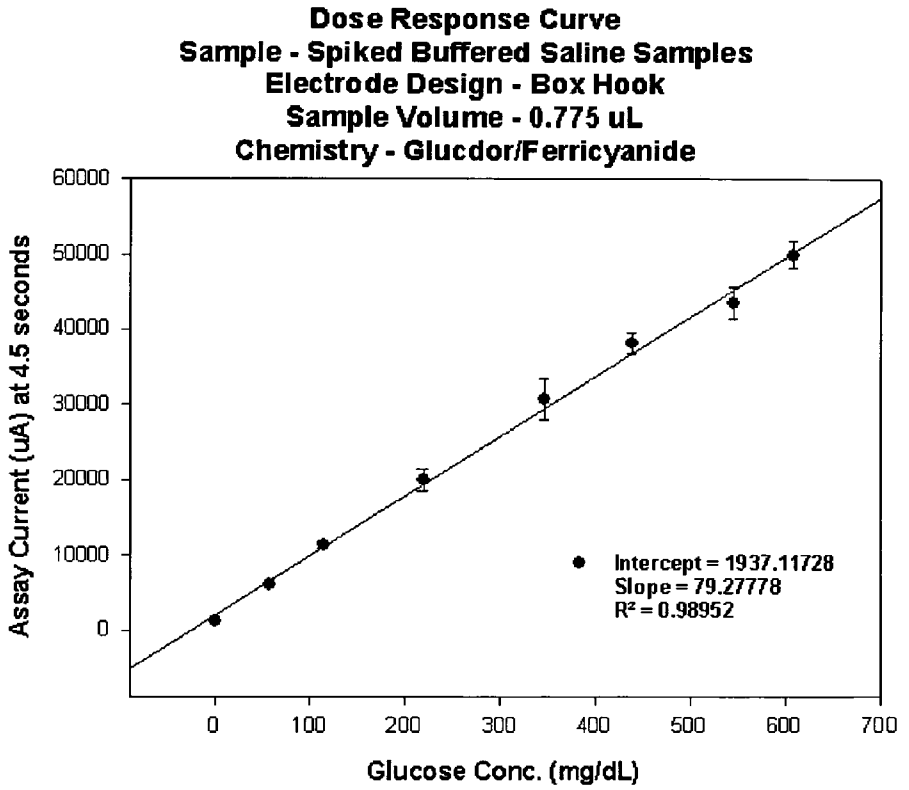

Dose Response Curve
Sample - Spiked Buffered Saline Samples
Electrode Design - Box Hook
Sample Volume - 0.775 uL
Chemistry - Glucdor/Ferricyanide
Assay Current (uA) at 4.5 seconds
60000
50000
40000
30000
20000
10000
0
0
100
200
300
400
500
600
700
Glucose Conc. (mg/dL)
Intercept = 1937.11728
Slope = 79.27778
R² = 0.98952

Fig. 10

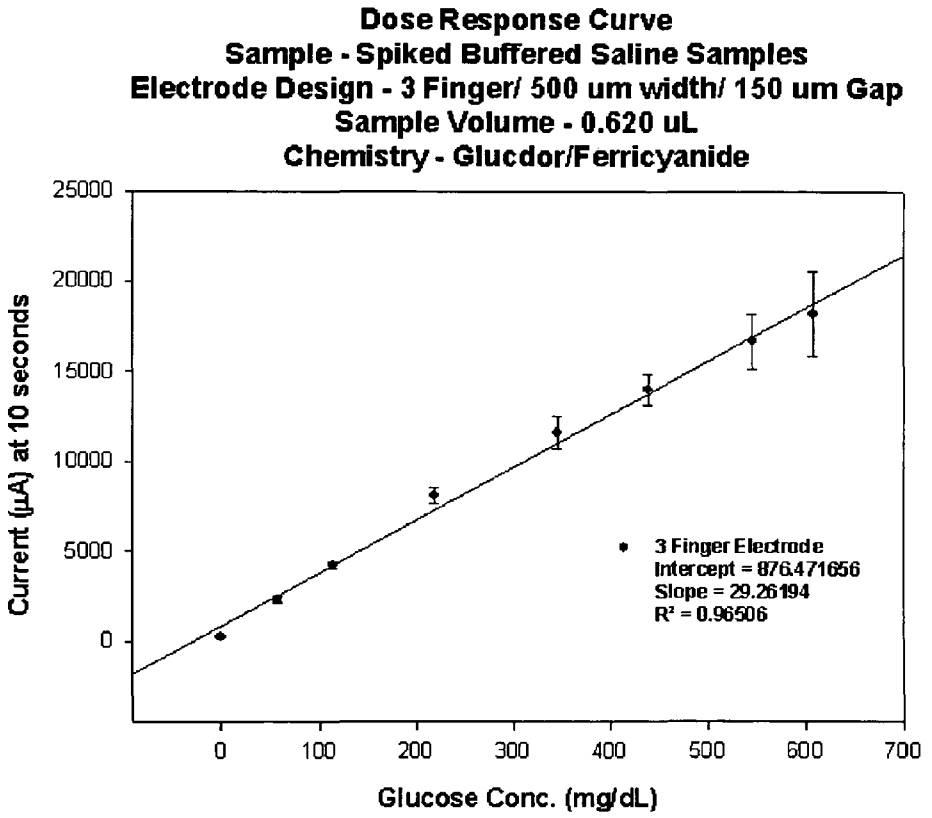

Dose Response Curve
Sample - Spiked Buffered Saline Samples
Electrode Design - 3 Finger/ 500 um width/ 150 um Gap
Sample Volume - 0.620 uL
Chemistry - Glucdor/Ferricyanide
Current (µA) at 10 seconds
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
600
700
Glucose Conc. (mg/dL)
3 Finger Electrode
Intercept = 876.471656
Slope = 29.26194
R² = 0.96506

Fig. 11

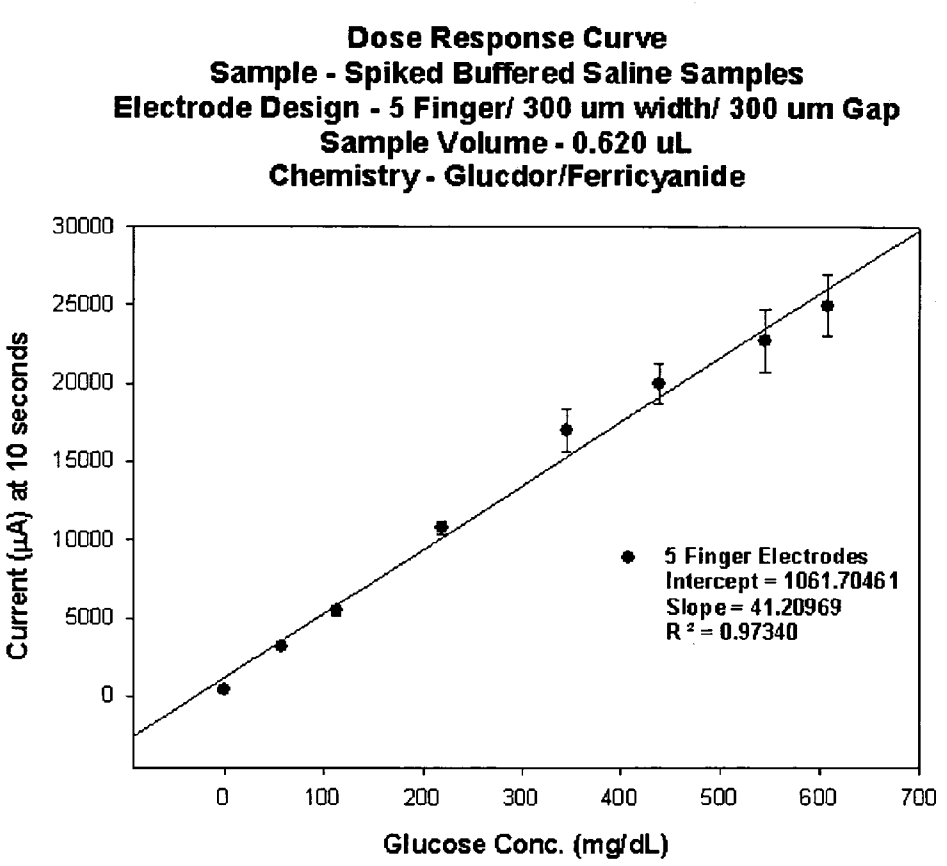

Dose Response Curve
Sample - Spiked Buffered Saline Samples
Electrode Design - 5 Finger/ 300 um width/ 300 um Gap
Sample Volume - 0.620 uL
Chemistry - Glucdor/Ferricyanide
Current (µA) at 10 seconds
30000
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
600
700
Glucose Conc. (mg/dL)
5 Finger Electrodes
Intercept = 1061.70461
Slope = 41.20969
R ² = 0.97340

Fig. 12

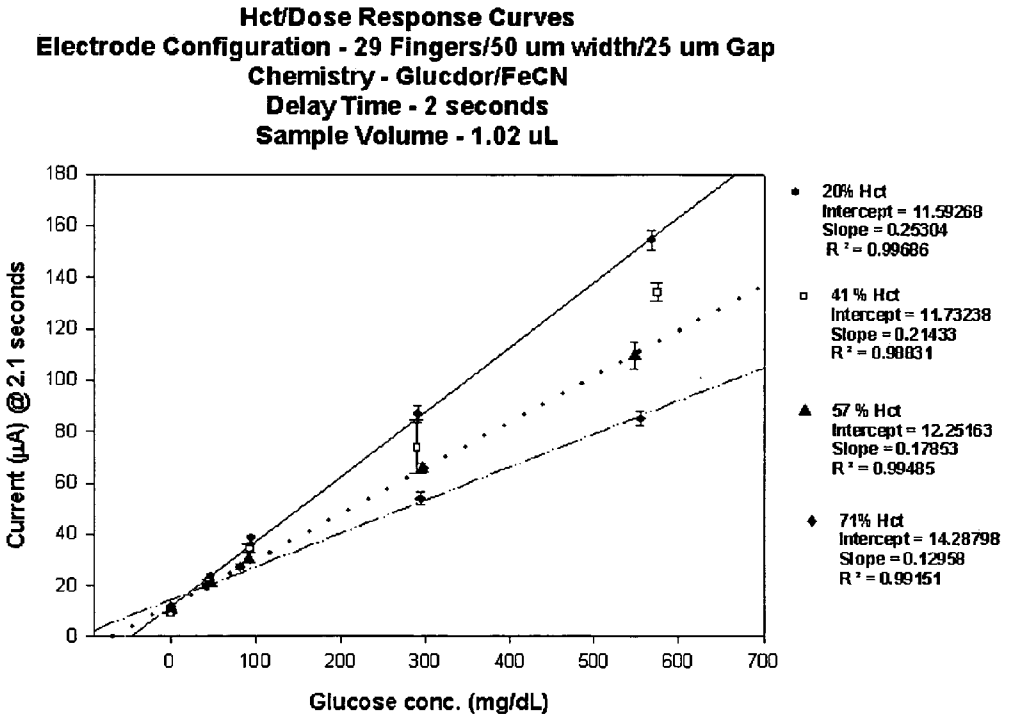

Hct/Dose Response Curves
Electrode Configuration - 29 Fingers/50 um width/25 um Gap
Chemistry - Glucdor/FeCN
Delay Time - 2 seconds
Sample Volume - 1.02 uL
Current (µA) @ 2.1 seconds
Glucose conc. (mg/dL)
0
20
40
60
80
100
120
140
160
180
0
100
200
300
400
500
600
700
20% Hct
Intercept = 11.59268
Slope = 0.25304
R² = 0.99686
41 % Hct
Intercept = 11.73238
Slope = 0.21433
R² = 0.98831
57 % Hct
Intercept = 12.25163
Slope = 0.17853
R² = 0.99485
71% Hct
Intercept = 14.28798
Slope = 0.12958
R² = 0.99151

Fig. 13

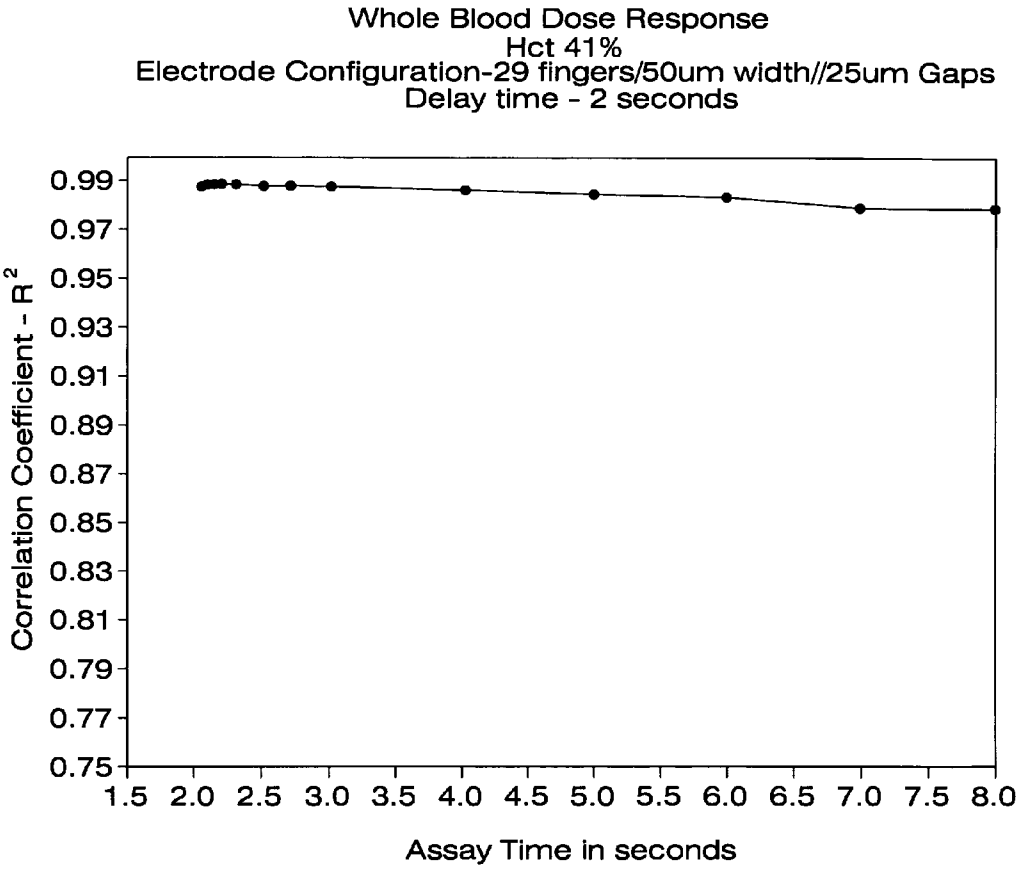

Whole Blood Dose Response
Hct 41%
Electrode Configuration-29 fingers/50um width//25um Gaps
Delay time - 2 seconds
Correlation Coefficient - R^2
0.99
0.97
0.95
0.93
0.91
0.89
0.87
0.85
0.83
0.81
0.79
0.77
0.75
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0
Assay Time in seconds

Fig. 14

ELECTRODES, METHODS, APPARATUSES COMPRISING MICRO-ELECTRODE ARRAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Ser. No. 60/332,411 filed on Nov. 16, 2001, which is hereby incorporated by reference in its entirety

FIELD OF THE INVENTION

The present invention relates to arrays of micro-electrodes, to methods of preparing the arrays, and to uses of the arrays. The arrays may be used in conventional applications for electrodes. In one embodiment of the invention, the array is an interdigitated array, and may be used as an electrode in an electrochemical sensor.

BACKGROUND

Electrodes are well known devices which permeate industry, and which, although often very small in size and not particularly visible, can have a significant impact on peoples' lives. Electrodes are used in electronic instruments having many industrial, medical, and analytical applications. To name just a few, they include monitoring and controlling fluid flow, and various types of analytical methods wherein electric current is measured to indicate the presence or concentration of certain chemical species.

With respect to analytical methods, the need for detection and quantitative analysis of certain chemicals found within a larger composition can be important for the chemical and manufacturing industries, as well as biotechnology, environmental protection, and health care industries. Examples of substances that may be analyzed include liquid samples such as tap water, environmental water, and bodily fluids such as blood, plasma, urine, saliva, interstitial fluid, etc.

Many analytical techniques, sometimes referred to as electrochemical detection methods, make use of electrodes as a component of an electrochemical sensor. The sensors are used in combination with electronic apparatuses to precisely detect the presence or concentration of a selected chemical species (analyte) within a substance sample. Techniques that allow the use of miniaturized disposable electroanalytic sample cells for precise micro-aliquote sampling, and self-contained, automatic means for measuring the analysis, can be particularly useful.

Electrochemical detection methods can include amperometric measurement techniques, which generally involve measurement of a current flowing between electrodes that directly or indirectly contact a sample of a material containing an analyte, and studying the properties of the current. The magnitude of the current can be compared to the current produced by the system with known samples of known composition, e.g., a known concentration of analyte, and the quantity of analyte within the sample substance can be deduced. These types of electrochemical detection methods are commonly used because of their relatively high sensitivity and simplicity.

Micro-electrode arrays are structures generally having two electrodes of very small dimensions, typically with each electrode having a common element and electrode elements or micro-electrodes. If "interdigitated" the arrays are arranged in an alternating, finger-like fashion (See, e.g., U.S. Pat. No. 5,670,031). These are a sub-class of micro-electrodes in general. Interdigitated arrays of micro-electrodes, or IDAs, can exhibit desired performance characteristics; for example, due to their small dimensions, IDAs can exhibit excellent signal to noise ratios.

Interdigitated arrays have been disposed on non-flexible substrates such as silicon or glass substrates, using integrated circuit photolithography methods. IDAs have been used on non-flexible substrates because IDAs have been considered to offer superior performance properties when used at very small dimensions, e.g., with feature dimensions in the 1–3 micrometer range. At such small dimensions, the surface structure of a substrate (e.g., the flatness or roughness) becomes significant in the performance of the IDA. Because non-flexible substrates, especially silicon, can be processed to an exceptionally smooth, flat, surface, these have been used with IDAs.

SUMMARY OF THE INVENTION

Whereas micro-electrodes have in the past been used with non-flexible substrates such as silicon, ceramic, glass, aluminum oxide, polyimide, etc., it has now been discovered that micro-electrode arrays, for example, IDAs, can be advantageously useful when disposed on flexible substrates. Moreover, such micro-electrodes, disposed on flexible surfaces, can be prepared using methods that involve flexible circuit photolithography, as opposed to methods relating to integrated circuit photolithography.

An interdigitated array of the invention, disposed on a flexible substrate, can be used generally, in applications where IDAs are known to be usefully employed. In particular embodiments of the invention, the IDAs can be used to construct electrochemical sensors, test cells, or test strips. The sensors can be used with electronic detection systems (sometimes referred to as "test stands") in methods of analyzing sample compositions for analytes. Preferred embodiments of sensors can be disposable, and can include channels or microchannels, preferably a capillary, which facilitates flow of a substance sample into the reaction chamber and in contact with the sensor.

The micro-electrode arrays of the invention can be useful when disposed onto a flexible substrate. In particular, IDAs are shown to be effective at dimensions relatively larger than the dimensions often used for IDAs disposed on non-flexible substrates. Even though they can be relatively larger than IDAs disposed on non-flexible substrates, the inventive IDAs are still able to exhibit performance properties, e.g., signal to noise amplification benefits and steady-state assay profiles, comparable to IDAs having smaller dimensions.

Electrochemical sensors of the invention have been found to provide performance advantages, e.g., relative to commercially available sensors. For sensors used in glucose monitoring, compared to commercially available sensors, the inventive sensors can exhibit improved (shortened) processing periods, e.g., one half second to steady-state after application of the assay potential and 5 seconds to readout, and the ability to get an accurate and precise readout from a relatively small sample of substance, e.g., less than one microliter (μl), preferably a sample volume in the range from about 0.25 to 1.0 μl, e.g., from about 0.4 to about 1.0 μl.

The use of larger-dimensioned micro-electrode arrays also allows the significant advantage of fabricating arrays and sensors using relatively less expensive and more efficient flex circuit photolithography processes. These can advantageously incorporate the use of solid materials instead of spin-on liquid materials, e.g., one or more of a solid

photoresist or a solid coverlay, instead of liquid materials typically used in integrated circuit photolithography.

An aspect of the invention relates to micro-electrodes used in combination with a flexible substrate. The array can include a working electrode and a counter electrode, each including a common lead and commonly-connected electrode elements, for example with the electrode elements being arranged in a substantially-parallel, alternating fashion. Preferred dimensions for micro-electrodes can be, e.g., feature size or width of electrodes (W_e) in the range from 15 or 20 or 25 μm, up to about 100 μm, more preferably from greater than or about 25 or 30 μm to about 50 μm. Preferred spacing between electrodes (W_g) can also be in the range from about 15 to about 50 μm, more preferably from greater than or about 20 or 25 μm to about 45 μm.

Another aspect of the invention relates to an electrochemical sensor comprising an array of micro-electrodes disposed on a flexible substrate. The sensor can further include a chemical coating disposed on the array to facilitate practice of electrochemical detection methods.

Yet another aspect of the invention relates to a method of detecting an analyte using an array of micro-electrodes of the invention, e.g., using an electrochemical sensor comprising an interdigitated array disposed proximal to a flexible substrate. Such a method can include certain of the following steps. A sensor is provided which comprises micro-electrodes proximal to a flexible substrate, and a chemical coating proximal to the micro-electrodes; the coating comprises a compound reactive to produce an electroactive reaction product. The coating is contacted with a substance comprising an analyte, allowing the analyte to react with chemical components of the coating to produce an electroactive reaction product. Electric properties of the coating can be measured, and the electric properties can be correlated to the amount of electroactive reaction product, and to the amount of analyte.

Still another aspect of the invention relates to a method of preparing a micro-electrode, including the step of disposing the micro-electrode onto a flexible substrate.

More particularly, the present invention comprises a method for determining the concentration of glucose in a blood sample. The method utilizes a disposable test strip having a capillary-fill chamber including a working electrode and a counter and/or reference electrode and a reagent. The reagent includes an enzyme and a mediator, and reacts with glucose to produce an electroactive reaction product. The method involves providing a blood sample to the capillary chamber, detecting the presence of the blood sample in the capillary chamber, and thereafter applying or controlling the voltage or current across the working and counter electrodes. Within 10 seconds of detecting the presence of the blood sample, the glucose concentration is determined.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows an embodiment of an interdigitated array of electrodes in accordance with the invention.

FIGS. **2** and **2A** each show a top view of a sensor of the invention.

FIG. **3** shows a top view of a sensor of the invention.

FIG. **4** shows a side view of a sensor of the invention.

FIG. **5** shows an end view of a sensor of the invention.

FIG. **6** shows a perspective view of a dissembled sensor of the invention.

FIG. **7** shows a dose response plot of assay current versus blood glucose level.

FIG. **8** shows a Hct/dose response plot for glucose collected at 4.5 seconds after dose detection.

FIG. **9** shows a top plan view of an alternative embodiment of a pair of electrodes in accordance with the invention.

FIG. **9A** shows a top plan view of an alternative embodiment of a sensor incorporating the electrode pair of FIG. **9**.

FIG. **10** shows a dose response plot for glucose spiked saline samples collected at 4.5 seconds after dose detection.

FIG. **11** shows a dose response plot for glucose spiked saline samples collected at 10 seconds after dose detection.

FIG. **12** shows a dose response plot for glucose spiked saline samples collected at 10 seconds after dose detection.

FIG. **13** shows a Hct/dose response plot for glucose spiked whole blood samples collected at 2.1 seconds after dose detection.

FIG. **14** shows a plot of the correlation coefficient (r^2) versus assay time for the data collected in FIG. **13**.

DETAILED DESCRIPTION

An embodiment of the present invention is directed to arrays of micro-electrodes, e.g., an interdigitated array of electrodes (sometimes referred to as "microband" electrodes) used in combination with a flexible substrate.

An array of micro-electrodes includes two electrodes, referred to as the working electrode and the counter electrode, electrically insulated from one another.

Micro-electrodes, as distinguished from other electrodes generally, are understood in the electronic and biosensor arts. In analyzing a liquid sample using electrodes and electronic equipment and techniques, the size and spacing of electrodes can affect whether diffusion of an analyte through the sample to an electrode occurs by a planar or non-planar path. Micro-electrode arrays are of a size and spacing such that in detecting chemical species of a solution, the species will diffuse toward or approach an electrode of the micro-electrode array in a non-planar fashion, e.g., in a curved or hemispherical path of diffusion. In contrast, non-microclectrodes, i.e., "macro-electrodes," cause diffusion of an analyte through a solute according to a substantially planar path. It is also understood that some electrode configurations can cause diffusion to take place by a mix of planar and non-planar paths, in which case the electrodes can be considered a micro-electrode array, especially if the diffusion occurs predominantly (e.g., greater than 50%) according to a non-planar path, or if the size of the electrodes is less than 100 μm, e.g., less than 50 μm.

The electrodes of a micro-electrode array are positioned near each other in an arrangement that will result in non-planar diffusion as described. The arrangement of the electrodes can be any arrangement that results in such diffusion, with a working and a counter electrode being substantially evenly spaced from each other. One electrode may be arranged into a shape or figure or outline that will produce interstices within which the second electrode may be placed. For instance, one electrode can be arranged as an increasing radius, substantially circular spiral, with a continuous, long and narrow interstitial area being created between each successively larger revolution of electrode. The other electrode can be positioned in the interstitial area between revolutions, while the electrodes remain insulated from one another. The width and spacing of the electrodes can be arranged to result in micro-electrode array performance.

According to other forms of such micro-electrode arrays, the spiral may not be substantially circular, but could include linear, square, angled, or oblong or oval features. Or, the

electrodes could be arranged in any other geometric form whereby the electrodes are placed adjacent to each other and within the other's respective interstitial area, e.g., by following a similar path separated by a substantially uniform gap.

In one particular embodiment, the micro-electrode can be arranged into an interdigitated array, meaning that at least a portion of electrode elements of the working electrode are placed substantially parallel to and in alternating succession with at least a portion of the electrode elements of the counter electrode, e.g., in an alternating, "finger-like" pattern. Such interdigitated micro-electrode arrays include electrode elements (sometimes referred to as "fingers") and a common element ("contact strip") which commonly connects the electrode elements.

The components of the electrodes may be made of any conductive material, including those known and conventionally used as electrode materials, particularly including materials known in the flexible circuit and photolithography arts. These can include, for example, carbon, noble metals such as: gold, platinum, palladium, alloys of these metals, potential-forming (conductive) metal oxides and metal salts, as well as others.

The electrodes and their components can be of dimensions, meaning the width of the electrode components as well as the separation between components, that can provide an array with useful properties, e.g., useful or advantageous capabilities with respect to contacting a substance or measuring electrical properties. Advantageously, interdigitated arrays can be prepared at dimensions that allow for contact with and measurement of electrical properties of a relatively small sample of a substance.

In preferred embodiments of the invention, each electrode element can independently have a width (W_e) in the range from greater than 15 micrometers (μm) to about 50 μm, with the range from greater than or about 20 or 25 μm to about 40 μm being particularly preferred. The separation between electrode components (W_g), especially the separation between alternating electrode elements, can also preferably be in the range between about 15 micrometers and about 50 μm, with the range from greater than or about 20 or 25 μm to about 40 μm being particularly preferred. The total area of an electrode (meaning the area of the fingers but not the common element) can be chosen depending on these dimensions, on the use intended for the electrode, on the desired current level intended to pass through the electrode, and on the desired number of electrode elements. An exemplary area of an electrode having 10 electrode elements can be in the range from about 0.1 to about 0.5 square millimeters, (for example 10 electrode fingers having dimensions of 50 μm by 1 mm), e.g., from about 0.2 to 0.3.

The thickness of the electrode components can be sufficient to support a desired electric current. Exemplary thicknesses can be in the range from about 30 to 200 nanometers (nm), with a preferred thickness being about 100 nm.

The electrodes can independently have a number of interdigitated electrode elements sufficient to provide utility, e.g., allowing contact with a substance to measure its electrical behavior. Conventionally, the array can have substantially the same number (equal, plus or minus one) of electrode elements in the working electrode as are in the counter electrode, allowing the electrode elements to be paired next to each other in an alternating sequence. In some preferred embodiments of the array, such as in some of the applications described below for electrochemical sensors, each electrode of an array may typically have from about 4 to about 30 electrode elements.

FIG. **1** illustrates an embodiment of an array of the invention. Working electrode **2** and counter electrode **4** are arranged as an interdigitated array on flexible substrate **10**. (The figure is not to scale and its dimensions, as well as the dimensions of the other figures, should not be construed to limit the invention). The working and counter electrodes include common strips **6***a* and **6***b*, respectively, which can be connected to electrically conductive means (e.g., "connectors," "pads," or "leads," etc.) for connecting the electrodes to an external circuit. In the illustrated example, the working electrode includes electrode elements **8***a* connected to common strip **6***a*, and the counter electrode includes electrode elements **8***b* connected to common strip **6***b*.

According to the invention, the interdigitated array is disposed proximal to, e.g., on, a flexible substrate. To act as a flexible substrate, a material must be flexible and also insulating, and is typically relatively thin. The substrate should be capable of adhering components of an IDA, or additional components of a sensor, to its surface. Such thin, insulative, flexible substrates are known in the art of flexible circuits and flex circuit photolithography. "Flexible substrates" according to the present disclosure can be contrasted to non-flexible substrates used in integrated circuit (IC) photolithography but not in flexible circuit photolithography. Examples of non-flexible substrates used in IC photolithography include silicon, aluminum oxide, and other ceramics. These non-flexible substrates are chosen to be processable to a very flat surface. Typical flexible substrates for use in the invention are constructed of thin plastic materials, e.g., polyester, especially high temperature polyester materials; polyethylene naphthalate (PEN); and polyimide, or mixtures of two or more of these. Polyimides are available commercially, for example under the trade name Kapton®, from I.E. duPont de Nemours and Company of Wilmington, Del. (duPont). Polyethylene naphthalate is commercially available as Kaladex®, also from duPont. A particularly preferred flexible substrate is 7 mil thick Kaladex® film.

Interdigitated arrays of the invention can be used in applications generally known to incorporate electrodes, especially applications known to involve interdigitated arrays of electrodes. Various applications are known in the arts of electronics and electrochemistry, including applications relating to process and flow monitoring or control, and chemical analytical methods. The arrays may be particularly useful as a component of an electrochemical sensor, where there is added value, benefit, or cost efficiency, to the use of a flexible substrate, or where there is value, benefit, or cost efficiency in having an interdigitated array of dimensions relatively larger than the dimensions of interdigitated arrays conventionally disposed on non-flexible substrates.

An interdigitated array of the invention can, for example, be included in an electrochemical sensor (sometimes referred to as a "biosensor" or simply "sensor") used in electrochemical detection methods. Electrochemical detection methods operate on principles of electricity and chemistry, or electrochemistry, e.g., on principles of relating the magnitude of a current flowing through a substance, the resistance of a substance, or a voltage across the substance given a known current, to the presence of a chemical species within the substance. Some of these methods can be referred to as potentiometric, chronoamperometric, or impedence, depending on how they are practiced, e.g., whether potential difference or electric current is controlled or measured. The methods and sensors, including sensors of the invention, can measure current flowing through a substance due directly or indirectly to the presence of a particular chemical compound

(e.g., an analyte or an electroactive compound), such as a compound within blood, serum, interstitial fluid, or another bodily fluid, e.g., to identify levels of glucose, blood urea, nitrogen, cholesterol, lactate, and the like. Adaptations of some electrochemical methods and electrochemical sensors, and features of their construction, electronics, and electrochemical operations, are described, for example, in U.S. Pat. Nos. 5,698,083, 5,670,031, 5,128,015, and 4,999,582, each of which is incorporated herein by reference.

Oftentimes, a compound of interest (analyte) in a substance is not detected directly but indirectly, by first reacting the analyte with another chemical or set of chemicals proximal to or in contact with an IDA. The reaction produces an electroactive reaction product that is electrochemically detectable and quantifiable by applying a potential difference between the counter and working electrodes and measuring the magnitude of the current produced. This allows measurement of the amount of electroactive reaction product generated by the first reaction, and correlation of that measurement to the amount of analyte in the sample substance.

An example of such a method involves the catalytic use of an enzyme, and is sometimes referred to as enzymatic amperometry. These methods can use an interdigitated array of electrodes coated with a chemical coating that contains a chemical compound reactive to produce an electroactive reaction product. (The chemical compound reactive to produce an electroactive reaction product is sometimes referred to herein as a "mediator.") Upon contacting the coating with a sample that contains an analyte, analyte reacts with chemical compounds of the coating to generate electroactive reaction product. This electroactive reaction product can be electronically detected, measured, or quantified, by applying a potential difference between the electrodes and measuring the current generated by the electrooxidation of the mediator at the working electrode. By calibrating the system's behavior using known substances and concentrations, the electrical behavior of the system in the presence of a sample substance of unknown composition can be determined by comparison to the calibration data.

The sensor of the invention may be used in amperometric applications, e.g., enzymatic amperometric applications, if disposed on the array is a coating of useful chemistry, including e.g., an enzyme and a mediator. When a sample containing an analyte is contracted with the coating, the analyte, enzyme, and the mediator participate in a reaction, wherein the mediator is either reduced (receives at least one electron) or is oxidized (donates at least one electron). Usually, in this reaction, the analyte is oxidized and the mediator is reduced. After this reaction is complete, an electrical potential difference can be applied between the electrodes. The amount of reducible species and the applied potential difference must be sufficient to cause diffusion-limited electrooxidation of the reduced form of the mediator at the surface of the working electrode. The IDA electrode configuration of the sensor places the working electrode fingers in close proximity to counter electrode fingers. Mediator electrooxidized at the working electrode can therefore diffuse rapidly to the adjacent counter electrode via radial diffusion where it is once again reduced. Likewise, oxidized mediator reduced at the counter electrode can migrate to the working electrode for electrooxidation to the oxidized form. This migration between the fingers produces a constant or "steady state" current between the electrodes. After a short time delay, this steady state current is measured and correlated to the amount of analyte in the sample.

The chemistries of the first and second reactions can be of any nature effective to produce the electroactive reaction product of the first reaction, to detect or quantify the electroactive reaction product during the second reaction, and to allow correlation of the amount of electroactive reaction product with the presence or concentration of analyte in the original sample.

In general, a typical first reaction can be an oxidation/reduction sequence, preferably occurring without the need for a chemical potential across the electrodes. It can be desirable for this reaction to favor maximum, preferably complete conversion of the analyte, and to proceed as quickly as possible. Often this reaction is catalyzed, e.g., enzymatically. Such reaction schemes and their application to enzymatic amperometry are known. See, e.g., U.S. Pat. No. 5,128,015; European Patent Specification EP 0 406 304 B1; and Aoki, Koichi, Quantitative Analysis of Reversible Diffusion-Controlled Currents of Redox Soluble Species at Interdigitated Array Electrodes Under Steady-State Conditions, J. Electroanal. Chem. 256 (1988) 269–282. An example of a useful reaction scheme can be the reaction of a component of a bodily fluid, e.g., glucose, with an enzyme and a cofactor, in the presence of a mediator, e.g., an oxidizer, to produce an electroactive reaction product.

The chemistry of a first reaction scheme of any chosen electrochemical detection method can be chosen in light of various chemical factors relating to the system, including the identity of the analyte and of the sample substance. Even then, for a given analyte or substance, various different reactive components may be useful in terms of a catalyst (often, a variety of enzymes will be useful), co-reactants (e.g., a variety of mediators may be useful), and cofactors (if needed, a variety may be useful). Many such reaction schemes and their reactive components and reaction products are known, and examples of a few different enzymes include those listed in Table 1.

TABLE 1

Analyte	Enzymes	Redox Mediator (Oxidized Form)	Additional Mediator
Glucose	Glucose dehydrogenase and Diaphorase	Ferricyanide, osmium (III)-(bipyridyl)-2-imidazolyl-chloride, Meldola blue, $[Ru(NH_3)_5MeIm]Cl_3$ [OS(III) $(NH_3)_5pyz]_2(SO_4)_3$, NITROSO analine derivatives	

TABLE 1-continued

Analyte	Enzymes	Redox Mediator (Oxidized Form)	Additional Mediator
Glucose	Glucose oxidase	(see above)	
Cholesterol	Cholesterol esterase and Cholesterol oxidase	(see glucose)	2,6-Dimethyl-1, 4-Benzoquinone, 2,5-Dichloro-1, 4-benzoquinone, or phenazine ethosulfate
HDL Cholesterol	Cholesterol esterease and Cholesterol oxidase	(see glucose)	2,6-Dimethyl-1, 4-Benzoquinone, 2,5-Dichloro-1, 4-benzoquinone, or phenazine ethosulfate
Triglycerides	Lipoprotein lipase, Glycerol kinase, Glycerol-3-phosphate oxidase	(see glucose)	Phenazine methosultate, phenazine ethosulfate.
Triglycerides	Lipoprotein lipase, Glycerol kinase, Glycerol-3-phosphate dehydrogenase and Diaphorase	(see glucose)	Phenazine methosultate, phenazine ethosulfate.
Lactate	Lactate oxidase	(see glucose)	2,5-Dichloro-1, 4-benzoquinone
Lactate	Lactate dehydrogenase and Diaphorase	(see glucose)	
Lactate Dehydrogenase	Diaphorase	(see glucose)	
Pyruvate	Pyruvate oxidase	(see glucose)	
Alcohol	Alcohol oxidase	(see glucose)	
Alcohol	Alcohol dehydrogenase and Diaphorase	(see glucose)	
Uric acid	Uricase	(see glucose)	
3-Hydroxybutric acid (ketone bodies)	3-Hydroxybutyrate dehydrogenase and Diaphorase	(see glucose)	

A mediator can be any chemical species (generally electroactive), which can participate in a reaction scheme involving an enzyme, an analyte, and optionally a cofactor (and reaction products thereof), to produce a detectable electroactive reaction product. Typically, participation of the mediator in this reaction involves a change in its oxidation state (e.g., a reduction), upon interaction with any one of the analyte, the enzyme, or a cofactor, or a species that is a reaction product of one of these (e.g., a cofactor reacted to a different oxidation state). A variety of mediators exhibit suitable electrochemical behavior. A mediator can preferably also be stable in its oxidized form; may optionally exhibit reversible redox electrochemistry; can preferably exhibit good solubility in aqueous solutions; and preferably reacts rapidly to produce an electroactive reaction product. Examples of suitable mediators include benzoquinone, medula blue, other transition metal complexes, potassium ferricyanide, and nitrosoanalines, see U.S. Pat. No. 5,286, 362. See also Table 1.

To describe an example of an oxidation/reduction reaction scheme that is known to be useful for detecting glucose in human blood, a sample containing glucose can react with an enzyme (e.g., Glucose-Dye-Oxidoreductase (Gluc-Dor)) and optionally a cofactor, (e.g., pyrrolo-quinoline-quinone), in the presence a redox mediator (e.g., benzoquinone, ferricyanide, or nitrosoanaline derivatives), to produce the oxidized form of the analyte, gluconolactone, and the reduced form of the redox mediator. See U.S. Pat. No. 5,128,015. Other examples of reaction schemes are known, and are typically used in methods designed to detect a specific analyte, e.g., cholesterol, urea, etc.

After the reaction is complete, a power source (e.g., battery) applies a potential difference between the electrodes. When the potential difference is applied, the amount of oxidized form of the redox mediator at the counter electrode and the potential difference must be sufficient to cause diffusion-limited electrooxidation of the reduced form of the redox mediator at the working electrode surface. In this embodiment, the close proximity of the counter and working electrode fingers in the IDA electrode configuration aids in the fast radial diffusion of the reduced and oxidized redox mediator between the electrodes. Recycling of the mediator between the electrodes and their subsequent oxidation and reduction on the electrodes generates a constant or "steady state" assay current. This steady state assay current is measured by a current measuring meter.

The measured current may be accurately correlated to the concentration of analyte in the sample when the following requirements are satisfied:

1) the rate of oxidation of the reduced form of the redox mediator is governed by the rate of diffusion of the reduced form of the redox mediator to the surface of the working electrode; and

2) the current produced is limited by the oxidation of the reduced form of the redox mediator at the surface of the working electrode.

In the preferred embodiment, these requirements are satisfied by employing a readily reversible mediator and by using a mixture of amounts of mediator and other components of the chemical layer to ensure that the current produced during diffusion limited electrooxidation is limited by the oxidation of the reduced form of the mediator at the working electrode surface. For current produced during electrooxidation to be limited by the oxidation of the reduced form of the mediator at the working electrode surface, the amount of reducible species at the surface of the counter electrode must always exceed the amount of the reduced form of the redox mediator at the surface of the working electrode.

An example of a reaction scheme relates to the detection of glucose using ferricyanide and Glucose-Dye-Oxidoreductase (Glur-Dor). The electroactive reaction product of the enzymatic reaction between glucose and the enzyme is the reduced mediator, ferrocyanide. The ferrocyanide is electrooxidized at the working electrode back to ferricyanide. One mole of oxidized redox mediator is reduced at the counter electrode for every mole of reduced redox mediator oxidized at the working electrode. Ferricyanide electrooxideized at the working electrode, diffuses to the counter electrode, and the ferrocyanide produced at the counter electrode can rapidly diffuse to the working electrode where it is again oxidized. A "quasi-steady state" concentration gradient is established between the counter and working electrode pairs resulting in generation of a constant quasi-steady state current at the working electrode.

The magnitude of the current, preferably as measured at a quasi-steady-state condition, can be correlated to the amount of electroactive reaction product present in the coating, and consequently, to the amount of analyte in the sample.

The chemical coating should allow diffusion of analyte into the coating, followed by reactions as described. The coating can include materials which can contain the reactive chemical components, which allow reaction between the components to product an electroactive reaction product, which allow necessary diffusion of chemical components, and which can support a current passing through the coating based on the concentration of electroactive reaction product. Typically, the coating can be made up of a binder that contains a set of chemicals which react to produce an electroactive reaction product. The chemicals generally include a mediator and necessary enzymes and cofactors. Such a coating can also contain a variety of additional components to make the coating operative and suitable for processing, including specific components listed above as well as surfactants, film formers, adhesive agents, thickeners, detergents, and other ingredients and additives that will be understood by an artisan skilled in the electrochemical sensor art.

The binder can provide integrity of the coating while allowing diffusion of the different components of the reaction scheme, reaction between the reactive components, and movement of reactive components and products sufficient to produce a quasi-steady-state concentration gradient of mediator and electroactive reaction product and thereby establish a stable or quasi-steady-state current between the electrode pairs. Exemplary binders can include gelatin, carrageenan, methylcellulose, polyvinyl alcohol, polyvinylpyrrolidone, alignate, polyethylene oxide, etc.

A sensor according to the invention can be understood to include a micro-electrode disposed on a flexible substrate, optionally including a chemical coating, and further including any immediate appurtenance necessary to use the sensor in an electronic system or apparatus (e.g., test stand) designed, for example, for use in an electrochemical detection method. A sensor can include the interdigitated array disposed on a flexible substrate, with additional components to independently connect each of the separate electrodes to a different voltage, e.g., electrical connectors, leads, or pads. In some circumstances, the sensor may include a reference electrode provided on the same or a different substrate and electrically insulated from the interdigitated array. The sensor may also include components to direct flow of a sample substance into contact with the IDA, e.g., a vessel, channel, microchannel, or capillary. A particularly preferred embodiment of the sensor includes a microchannel or capillary, most preferably a capillary, which directs flow of a sample substance into the reaction chamber and over the IDA (e.g., a coated IDA).

A capillary can be included in a sensor to facilitate analysis of a small volume of a sample substance by precisely directing the flow of a volume of sample over the IDA, preferably in a short period of time. Analysis of relatively small volumes of a sample substance can be accomplished, at least in part, due to the signal amplification features of the IDA.

Preferred dimensions of a capillary for what can be referred to as a "low volume sensor configuration," can be in the range of 0.025 mm to 0.2 mm (depth), preferably about 0.125 mm (depth), ×1 mm (width)×3 mm (length), resulting in a capillary chamber requiring a relatively small volume of sample, e.g., less than 400 nanoliters (nL). The volume of the chamber can preferably be such that a low volume sample of a substance can be directed into or through the chamber for analysis. Chamber volumes will vary depending on the type of analyte being studied, and even its concentration of an analyte. (Blood samples of different hematocrits will dispense differently into a capillary.) Exemplary chamber volumes can be in the range from about 100 to 300 nanoliters for glucose analysis in interstitial fluid, and from about 250 to 400 nanoliters for glucose analysis applications in the whole blood. In the most preferred embodiments of the sensor, including a capillary, the capillary may have a vent to facilitate flow of a sample substance into the capillary chamber by equalizing pressure between the interior and exterior of the chamber.

The sensor of the invention can include these and other features, and, especially if an embodiment is disposable, can be referred to as a "test strip" or a "test cell." The term "disposable" refers to sensors designed or sold for a single use, after which they are to be discarded or otherwise stored for later disposal.

Capillaries may be fabricated as a component of a sensor, using photolithographic methods, e.g., as described infra.

An example of a sensor construction is shown in FIG. **2**, according to the preferred embodiment. The figure shows sensor **20**, including an interdigitated array of electrodes **22** disposed on flexible substrate **24**. The electrodes are connected to electrically-conductive connectors **26** which include portions **28** that can be identified as pads, located on

the surface of the flexible substrate, where they are available to be contacted to an external electronic circuit such as a testing apparatus. The connectors also include connector portions **30**, which connect electrode elements at the array to the pads, and which may typically be covered by an insulating layer. FIG. **2***a* shows a close-up of array **22**, showing that electrodes attached to each of connectors **26** are arranged in an inter digitated fashion (as shown in FIG. **1**).

FIG. **3** shows different details of a sensor of the invention. FIG. **3** shows sensor **20** comprising flexible substrate **24**, an array of interdigitated electrodes **22**, and connectors and pads. Non-conductive layer **32** is disposed over the substrate and connector portions **30** of the connectors **26**, over portions of the array **22**, and not over a rectangular capillary portion including some of the substrate and an intersection of array **22**; this rectangular portion defines capillary chamber **34**. (A chemical coating, not shown in this figure, is preferably disposed over the array, within the capillary chamber.) Foil **36** covers a rectangular portion of the sensor, including portions of the non-conductive layer **32**, and a portion of capillary chamber **34**, except for air vent **38**. This embodiment is shown from one side in FIG. **4**, and from another side in FIG. **5**. FIG. **5** specifically illustrates substrate **24**, array **22**, non-conductive layer **32**, which defines chamber **34**, and foil **36**. FIG. **5** additionally includes coating **40** disposed on array **22**, within the capillary.

FIG. **6** illustrates an exploded view of a sensor of the invention. The sensor **20** includes flexible substrate **24**; a conductive film **40** patterned with an interdigitated array of electrodes **22** and connectors **26** which include pad portions **28** and connecting portions **30**, an insulating material **32** which defines the depth and dimensions of capillary chamber **34**, a chemical coating **40** disposed in the capillary chamber **34**, and top foil **36** coated with a hydrophilic adhesive layer **42**.

The array of the invention, in various embodiments such as a sensor, can be used in electrochemical detection methods, including those using the principles and specific methods described above, and others. Such methods employ the array disposed on a flexible substrate, preferably further including a chemical coating contacting the array.

Upon contacting the coating with a sample containing analyte, analyte generally diffuses into the coating at a rate dependant on factors such as the chemical composition of the coating and the chemical identity of the analyte. Generally, the chemical coating will be at least partly solubilized or hydrated by the sample substance. For a method to provide the quickest read time (the time following contact with a substance sample, when a reading of the concentration of analyte in the substance is available), it is desirable that the analyte diffuse quickly into the coating, and thereafter quickly and completely react to produce an electroactive reaction product. The period during which this occurs can be reduced by operating on a relatively small volume of sample, and by using a sensor having a relatively small amount of chemical coating to be solubilized or hydrated.

The time from when the substance containing the analyte is contacted with the chemical coating until an assay potential is applied to the array, and during which the analyte diffuses into the coating and reacts to produce an electroactive reaction product, can be referred to as the "delay period". This period can be any amount of time necessary for the above occurrences to transpire, is preferably minimized, and in some embodiments can be less than about 10 seconds, preferably in the range from about 2 to 6 seconds.

After a delay period, the electric properties of the coating can be measured. By chronoamperometric methods, or by potentiometric methods, as will be appreciated by the skilled artisan, either the current or the applied potential can be controlled, and any of the related current, resistance, or voltage can be measured and correlated to amounts of electroactive reaction product and analyte. The magnitude of the current, or alternatively potential difference or the resistance of the chemical coating, can be measured using an external circuit connected to the sensor electrodes.

As an example, according to chronoamperometric methods, a potential ("assay potential") can be applied across the electrodes, inducing a current ("assay current") to flow through the coating. The potential should be enough to cause reduction or oxidation of the redox products formed in the first step of a binary reaction scheme (e.g., as described above), but should not be sufficient to cause other electrochemical reactions or to otherwise cause significant current to flow through the coating. The assay potential can be chosen depending on the redox mediator chosen, factors relating to the electrochemical detection method, the electrochemical system and reaction scheme, and the general capabilities of the sensor. A typical potential can be in the range of a few to several hundred millivolts, e.g., from about 100 to 500, preferably 200 to 400 millivolts.

A measured current can initially exhibit a spike to a relatively elevated level, and can then descend to a steady-state current based on a quasi-steady-state concentration gradients and a recycle reaction loop of the mediator and electroactive reaction product. Preferably, the magnitude of the current can be measured at a time when current flowing through this system has approached a plateau, based on quasi-steady-state concentration gradients. The period of time starting with application of the assay potential and lasting to the plateau or near-steady-state current can be referred to as the "assay period." Steady-state assay currents can occur within various such time periods, depending upon the reaction scheme, the chemistries of its components, etc. In the practice of the invention, assay periods of less than one minute are preferred, e.g., less than 30 seconds, and assay periods of even shorter duration, less than 10 seconds, are most preferred. The assay profile (the profile of the assay current over time) can be to some extent controlled by changing the spacing between electrode elements in the array; increased spacing between electrode elements can result in a longer time interval between assay potential application and formation of the steady state assay currents.

Assay currents exhibited by exemplary sensors of the invention can be any current that will function in an electrochemical detection method. For the sensors of the invention, any useful current can be used, preferably with a range between a lower end in the nanoamp range (e.g., between 20 to 25 nanoamps) up to the microamp range e.g., 100 microamps, being an exemplary working range, e.g., at the steady state current plateau. Typical steady state assay currents can be in the range from below one microamp up to around 100 microamp, preferably from about 0.5 to about 25 microamps. In an embodiment of the invention useful for detecting glucose content of a blood sample, the current response (steady state assay current) in this range has been found to be linear with respect to the concentration of glucose in the sample, particularly for glucose concentrations in the range from about 0 to 600 milligrams per deciliter (mg/dL).

Sensors of the invention may be used in cooperation with electronic or computerized systems and apparatuses, and in combination with methods for identifying analytes and measuring concentration of analytes within a substance sample. For example, a sensor can be used with a VXI or

Biopotentiostat test stand built from components purchased from National Instrument Corp., Austin, Tex. In this context, the method of the invention can be practiced with a delay period of around 3 seconds, an assay potential of about 300 millivolts, and an assay period which, although variable, can preferably be in the range from about 1.5 to 2 seconds after applying the assay potential.

The sensors can be used in such a method to detect and quantify the concentration of an analyte within a sample substance. The analyte can be chosen from various chemical compounds present within any of a large variety of substances, generally fluids. Examples of analytes include glucose, cholesterol, urea, and the like. Examples of substances containing the analyte include bodily fluids such as blood, urine, and interstitial fluid; water such as environmental water, ground water, waste water, etc.

In some embodiments of the invention, analytes can be detected at very low concentrations, for example glucose can be measured at cocentrations as low as 0.5 mg/dL (5 ppm) in blood using ferricyanide as the mediator.

The use of an array or sensor of the invention offers certain practical advantages. For instance, a flexible substrate can be used in combination with relatively larger-dimensioned electrodes, including electrode components of increased size (e.g., width) as well as increased spacing between them. Lower sample volumes can independently decrease the time of the delay period. A shorter delay period in combination with an expedited formation of a quasi-steady-state region of the assay current produces a quicker read time. In the practice of the invention, read times of less than 10 seconds have been achieved, with a read times in the range from about 4 and 5 seconds being preferred.

Test cells and test strips according to the invention allow for controlled volumes of blood to be analyzed without pre-measuring. Insertion of the test cell into an electronic or computer-controlled apparatus (referred to generally as a test stand) permits automatic functioning and timing of the reaction and analysis of the sample. This allows for patient self-testing with a very high degree of precision and accuracy. The method, the sensor or test cell, and the apparatus, are designed to provide self-monitoring by a patient of important bodily fluids, e.g., blood glucose levels. The sensor is used to control the sample volume and reaction media, to provide precise, accurate, and reproducible analysis. Preferably, disposable test strips or test cells can be used in combination with a portable electrochemical testing meter.

The preferred embodiment of the present invention uses a micro-electrode array consisting of interdigitated micro-band electrodes as described above. Although this arrangement leads to the aforementioned re-cycling of redox products between narrowly separated working and counter electrodes, this is not a strict requirement for successful practice of the invention. An alternative embodiment is the provision of an array of more general micro-electrodes to act as the working electrode structure. These may be micro-bands that are not interdigitated with the counter electrode, or micro-disks, also not closely spaced with the counter electrode. In this case the width or diameter of the working electrode bands or disks should be of such a dimension as to allow for some degree of radial or spherical diffusion to the working electrode surfaces. Typically, this dimension should be in the range of 5 to 50 μm, and most preferably 10 to 50 μm for the case of aqueous systems such as encountered with a sensor used for the assay of biological fluids. In both cases the counter electrode is provided at a distance from the working array that is generally larger than the smallest dimension of the working electrodes.

In these embodiments, specific recycling of redox species between the working and counter electrodes is not observed in the same way as in other described embodiments, and assay current magnitudes are consequently reduced. Nevertheless, the effect of radial or spherical diffusion to working micro-electrode structures can still be observed as current densities that are greater than that predicted from linear diffusion alone. Although reduced in magnitude, and not approaching quasi-steady-state as displayed by the preferred embodiments, it is still possible to measure dose responses to the analyte in question (e.g. glucose) when the same reagent as described above is disposed on the micro-electrode array.

Micro-electrode arrays of the invention can be disposed onto a flexible substrate using various methods useful for disposing electronic components onto substrates, especially flexible substrates. A variety of such methods are generally known for fabrication of different types of circuitry, and include specific techniques of dry-coating, lamination, spin-coating, etching, and laser ablation. One or more of the following generalized methods may be specifically useful to prepare microelectrode arrays according to the invention.

One method of preparing a micro-electrode array as described herein, e.g., an IDA, is by the use of laser ablation techniques. Examples of the use of these techniques in preparing electrodes for biosensors are described in U.S. patent application Ser. No. 09/866,030, "Biosensors with Laser Ablation Electrodes with a Continuous Coverlay Channel" filed May 25, 2001, and in U.S. patent application Ser. No. 09/411,940, entitled "Laser Defined Features for Patterned Laminates and Electrode," filed Oct. 4, 1999, both disclosures incorporated herein by reference.

In general, laser ablative techniques use a laser to cut or mold a material. According to the invention, micro-electrodes can be prepared using ablative techniques, e.g., by ablating a multi-layer composition that includes an insulating material and a conductive material, e.g., a metallic laminate of a metal layer coated on or laminated to an insulating material. The metallic layer may contain pure metals or alloys, or other materials which are metallic conductors. Examples include aluminum, carbon (such as graphite), cobalt, copper, gallium, gold, indium, iridium, iron, lead, magnesium, mercury (as an amalgam), nickel, niobium, osmium, palladium, platinum, rhenium, rhodium, selenium, silicon (such as highly doped polycrystalline silicon), silver, tantalum, tin, titanium, tungsten, uranium, vanadium, zinc, zirconium, mixtures thereof, and alloys or metallic compounds of these elements. Preferably, the metallic layer includes gold, platinum, palladium, iridium, or alloys of these metals, since such noble metals and their alloys are unreactive in biological systems. The metallic layer may be any thickness but preferably is 10 nm to 80 nm, more preferably 20 nm to 50 nm.

In the laser ablation process, the metallic layer may be ablated into a pattern of micro-electrodes. The patterned layer may additionally be coated or plated with additional metal layers. For example, the metallic layer may be copper, which is then ablated with a laser, into an electrode pattern. The copper may be plated with a titanium/tungsten layer, and then a gold layer, to form desired micro-electrodes. Preferably, however, in some embodiments, only a single layer of gold is used. One example of a useful metallic laminate is a polyester or other flexible substrate such as a Kaladex film, coated with a layer of gold, preferably about 5 mils in thickness.

The conductive material is ablated with the laser to leave a micro-electrode array. Any laser system capable of ablation of the conductive material will be useful. Such laser systems are well known and commercially available. Examples include excimer lasers, with a pattern of ablation controlled by lenses, mirrors, or masks. A specific example of such a system is the LPX-400, LPX-300, or LPX-200, both from LPKF LASER ELECTRONIC, GMBH of Garbsen, Germany.

One specific example of a process for laser ablation is as follows. Sheets of sensor traces are fabricated in a MicrolineLaser 200-4 laser system (from LPKF). The system chamber includes a vacuum platen atop of a LPKF-HS precision positioning X,Y table, laser mirrors and optics, and a quartz/chromium photomask (International Phototool Company, Colorado Springs, Colo.) with the sensor components subdivided into rectangular fields on the mask. Photomask positioning, X,Y table movement and laser energy are computer controlled. Sheets of metal laminate 22 cm×22 cm in dimension are placed into the chamber onto the vacuum table. The table moves to the starting position and the Kr/F excimer laser (248 nm) is focused through the first field of the photomask onto the metal laminate. Laser light passing through the clear areas of the photomask field ablates the metal from the metal laminate. Chromium coated areas of the photomask block the laser light and prevent ablation in those areas, resulting in a metallized sensor structure on the laminate film surface. The complete structure of the sensor traces may require additional ablation steps through various fields on the photomask.

Another method of preparing the described micro-electrode array is the use of flex circuit photolithography. Flex circuit photolithography methods are well known. Two general methods of fabricating flexible circuits include the "additive" method and the "subtractive" method. With the additive method, an IDA and associated circuitry can be built up on top of a non-conductive flexible substrate. With the subtractive method, a non-conductive flexible substrate can be laminated with a conductive material (e.g., a copper foil) and conductive material is patterned using conventional photolithographic and wet chemical etching techniques. Some conventional processing steps include cleaning a substrate or intermediate; depositing conductive materials onto a substrate, e.g., by vapor deposition, electrodeposition, or vacuum plasma sputtering; depositing non-conductive or processing materials onto a substrate such as a photoresist material; masking and developing a photoresist material in a pattern defining an electrode; and removing excess developed or non-developed materials such as photoresist materials or conductive materials, to leave behind a desired arrangement of electrically conductive and insulating materials.

According to one series of steps in flex circuit photolithography, a substrate is prepared by cleaning, and a conductive material can be applied as a film to the substrate. Preferred thicknesses of a conductive layer (e.g., a gold conductive layer) can be in the range from about 500 to 1000 angstroms. It may be desirable to include a seed layer such as titanium or chromium between the conductive layer and the substrate, to improve adhesion. A preferred conductive material can be gold, and a preferred method of application can be sputtering, which has been found to provide very good adhesion.

A photoresist material can be applied to the conductive layer. Such photoresist materials are commercially known and available, with one example being Riston® CM206, from duPont. The thickness of the photoresist can be chosen to advantageously affect the resolution of the feature sizes of the electrode components. Improved resolution generally provides for better quality arrays, with fewer failures. There has been found a 1:1 relationship between the resolution of the smallest feature size achievable, and the thickness of the dry film photoresist, with thinner photoresist films providing better resolution (a thickness of about 0.6 mils generally allows a feature spacing or width of 0.6 mils). Riston® CM206, in the form of a 0.6 mil thick roll of film, can be a preferred photoresist because it can be capable of resolving features, i.e., lines and spaces, on a lower micron scale, e.g., in the range of 0.6 mils (15 microns) or lower. A photoresist layer often requires heating. Riston® CM206 does not require prebaking. The material is a dry film photoresist and is applied to the gold substrate using a heated laminated roller system. Once laminated, the material is ready for processing (exposure to UV light, and development).

The laminated film can be cut to a convenient size, e.g., one foot by one foot, and a pattern defining a micro-electrode array can be cured or crosslinked. This can generally be accomplished by conventional methods, e.g., using a mask pattern and exposing the array pattern to ultraviolet light, crosslinking the photoresist in the pattern of the array. Unexposed, uncrosslinked, photoresist can be developed away using a developing agent, which will typically be particular to the photoresist composition (e.g., lithium carbonate is one developing agent; see the manufacturer's instructions). At the end of this step, the substrate will have an undisturbed layer of the conductive material coated thereon, with a photoresist design defining the pattern of the array laid out on the conductive layer. This allows for unprotected conductive material to be etched away using an etchant (e.g., KI/I_2), to produce the IDA pattern in the conductive material. The remaining photoresist can then be removed.

Once an array is prepared, e.g., by laser ablative methods, using laminated dry photoresist, spin coating, etching, or other techinques, further processing of the micro-electrode array can be used to incorporate the array into a useful electronic device such as a biosensor. Preferably, additional materials can be disposed onto the array to form, for example, a spacer or insulating layer, optionally including a well or a microchannel or capillary. A well refers to a space over an array that defines the array. A microchannel or capillary more specifically refers to a space or channel that is defined over the array to allow the flow of a fluid over the array. The material used to define the microchannel or capillary can be any of a variety of materials useful insulating or spacing materials, sometimes referred to as "coverlay" materials, as well as other material useful for processing with the described fabrication methods. An example is Pyralux coverlay, and similar materials would also be useful.

Methods useful to place a microchannel or capillary onto the array include methods of mechanical lamination and mechanical removal of material to form a channel or capillary. One method would include a first step of mechanically "punching" (e.g., die punching) the coverlay material to cut away one or multiple portions of the material in the form of wells or channels, and then laminating the material to one or a number of sensors such that the channel is present over the array. Another method includes those types of methods generally referred to as "kiss die cutting" or "kiss cutting," which may be used to cut a well or channel in a coverlay layer, and then the coverlay material may be laminated onto the substrate with the well or channel over the array. One method of producing wells in a coverlay material is

described, for example, in U.S. patent application Ser. No. 60/332,192, entitled "Methods to Fabricate Biomedical Devices with Wells and Micro-Environments and Associated Products," filed on even date herewith, and having attorney docket No. 5051-552PR, the disclosure which is incorporated herein by reference.

A different example that includes a die punching method is as follows. A spacer foil was prepared by coating an adhesive, FastbondTM 30-NF Contact Adhesive to a wet thickness of 25 μm onto a 5 mil polyester film such as that sold under the trademark Melinex® S (DuPont Polyester films, Wilmington Del.) using a wire bar coater from Thomas Scientific of Swedesboro, N.J. The coated top foil was dried for 2 minutes at 50 C. in a horizontal air flow oven. The dried adhesive on the sheet was covered with either silicon or teflon release liner. Capillary channels and electrode contact well patterns were kiss cut into the sheet using an Aristomat 1310 ditigal die cutting system (Aristo Graphic Systeme GmbH & Co., Hambrug Germany). The spacer sheet can then be registered and laminated to an ablated sheet of sensor traces, as described above. Channels and electrode contact wells can also be produced using die punching processes in a similar fashion.

Another specific method by which to dispose a capillary or microchannel onto a micro-electrode array would be by methods of flex circuit photolithography. Accordingly, a photoimageable coverlay material such as Vacrel 8140®, (a dry film coverlay can be preferred) can be vacuum laminated onto the gold/plastic laminate. Multiple layers of various chosen thicknesses can be added to control the depth of the capillary chamber (see infra). The sheet can be exposed to ultraviolet light through a mask pattern to define capillary chambers. The exposed laminated sheet is developed by conventional methods, e.g., using 1% K_2CO_3, to remove crosslinked photopolymer coverlay material and leave behind components of a capillary. The sheet is generally thereafter cured, e.g., at 160 C. for 1 hour.

In fabricating the capillary, the depth of the chamber can be controlled by choosing the coverlay material or materials used, according to thickness. Vacrel 8140® film has a thickness of 2, 3, or 4 mil (100 μm). Pyralux PC® 1000, 1500, have 2000 have maximum thicknesses of 2.5 mils (63.5 μm), so double layer lamination gives a chamber depth of 127 μm. Pyralux 1010 has a thickness of 1 mil or 25.4 μm. Capillaries with depths of greater than or equal to 100 μm have been found to allow fast fill of blood with hematocrits from 20 to 70% to reliably flow into the chamber. Capillary depths of less than 100 microns to 25 microns can be used for other biological fluids such as serum, plasma, interstitial fluid, and the like.

A chemical coating may also be disposed onto the array. First, however, it may be beneficial to clean the sensors. By one cleaning method, a sheet of sensors as described can be plasma cleaned in a Branson/IPC Plasma Cleaner according to steps such as the following: (1) O_2 for 1 minute at 800 watts; (2) O_2/Argon(Ar) (70/30) for 3 minutes at 220 watts; (3) Ar for 2 minutes at 150 watts.

A chemical coating, as described, may be dispensed onto the array, e.g., into each capillary chamber and over the interdigitated arrays, by known methods. The method of dispensing is preferably capable of reproducibly and consistently delivering very small volumes of a chemical composition, onto the array—e.g., volumes in the range of hundreds of nanoliters, e.g., 625 nanoliters. As an example, such a coating may be dispensed using known syringe and metering techniques and apparatuses, including dispensing systems sold under the trade name Microdot (from Astro Dispense Systems, a DCI Company of Franklin, Mass. 02038-9908) and systems sold by BioDot Inc., Irvine, Calif. The coatings may then be dried of solvent. The inlet ports are opened, and a top foil coated with a hydrophilic adhesive is applied over the capillary chamber using heat and pressure to form the completed three-dimensional sensor structure.

The top foil can be any continuous film capable of defining one side of the capillary, and preferably being capable of appropriate processing, e.g., as described herein. Exemplary materials for the foil can include plastic films such as polyethylene naphthalate (PEN), film type Kadalex 1000, 7 mil thick.

Any of a variety of hydrophilic adhesives can be used to bond the top foil to the sensor. Two part thermoset adhesives such as polyurethane mixtures and isocyanate mixtures can be used, e.g., 38-8668 (polyurethane) and 38-8569 (isocyanate) (95:5 wt./wt.) from National Starch and Chemical Co. of Bridgwater N.J., or, a two part epoxy systems such as that sold under the trademark Scotch Weld™ 2216 B/A (3M Adhesive Div., St. Paul Minn.), as well as contact adhesives such as that sold under the trademark Fastbond™ 30-NF Contact Adhesive, provided that they exhibit acceptable sealing properties to the crosslinked coverlay surface. A preferred adhesive was found to be a mixture of Fastbond™ 30-NF Contact Adhesive and the surfactant Triton™ X-100 (Union Carbide, Danbury Conn.), 93%:7% wt./wt.

EXAMPLE 1

The following describes a process useful for preparing a sensor according to the invention, comprising an interdigitated array disposed on a flexible substrate. According to the method, a gold film can be deposited onto 7 mil thick Kaladex® film using a planar DC magnetron sputtering process and equipment, from Techni-Met Inc., Windsor, Conn. The thickness of the gold film can range from 30 to 200 nm, with a preferred thickness being 100 nm. Seed layers of chromium or titanium can be sputtered between the plastic film and the gold to promote better adhesion of the gold to the plastic substrate, however, gold layers sputtered directly onto the plastic film can exhibit sufficient adhesion.

The interdigitated array and connectors can be fabricated using batch photolithography processes common to the flex circuit industry. Electrodes with combinations of finger width and spacing between fingers in the range of 21 to 50 um were easily fabricated using these processes. A preferred configuration of the array was 21 total fingers (10 working electrode fingers and 11 counter electrode fingers), with finger dimensions of 25 microns (width) by 1 millimeter (length), with 21 micron spacing between the fingers.

After the gold was applied to the flexible substrate, a dry film photopolymer resist was laminated to the gold/plastic film. A dry film resist such as that sold under the trademark Riston® CM206 (duPont) was preferred. The Riston® CM206 photoresist was first wet laminated onto the gold surface of 12"×12" gold/plastic panels using a HRL-24 hot roll laminator (from duPont). The sealing temperature and lamination speed were 105° C. and 1 meter per minute. The laminated panel was placed in a Tamarack model 152R exposure system, from Tamarack Scientific Co., Inc., Anaheim, Calif. The release liner was removed from the top surface of the photoresist. A glass/emulsion photomask of the IDA configuration was produced by Advance Reproductions Corporation, North Andover, Mass. The emulsion side of the mask was treated with an antistick coating (Tribofilm Research Inc., Raleigh, N.C.), and was placed directly onto the photoresist surface of the panel. The laminated panel was

exposed to ultraviolet light of 365 nm through the photomask using an exposure energy of 60 mJ/cm^2. Exposed photoresist was stripped from the panel in a rotary vertical lab processor (VLP-20), Circuit Chemistry Equipment, Golden Valley, Minn., using 1% potassium carbonate, at room temperature, for 30 seconds using a nozzle pressure of 34 psi. Exposed gold on the sheet was then stripped using an etch bath containing a solution of: 4 parts I_2:1 part KI:40 parts water vol./vol.; and 0.04 gram Fluorad™ fluorochemical surfactant FC99, (3M, St. Paul, Minn.) per 100 gram solution, added to the bath to ensure wetting of the photoresist. Air was bubbled through the bath during the etch process to obtain uniform agitation of the bath mixture. The panel was rinsed with deionized water and residual Riston® CM206 was removed in a 3% KOH bath.

Sensor chambers were fabricated using dry film photoimageable coverlay materials such as that sold under the trademark Vacrel® 8140 (duPont) or Pyralux® PC series (duPont). The chamber dimensions can be accurately defined by flex circuit photolithography. Depth of the chamber was controlled by the thickness of the coverlay materials used, and whether single or multiple layers of the coverlay dry film were used. A preferred chamber depth was 125 microns (5 mil). This chamber depth was achieved by sequential lamination of different coverlay materials as follows: three mil thick Vacrel® 8130 was first laminated to the electrode side of the substrate using a HRL-24 (duPont) heated roll laminator at room temperature, using a roller speed of 1 meter per minute. The electrode panel was then vacuum laminated in a DVL-24 vacuum laminator (duPont) using settings of 120° F., 30 second vacuum dwell, and a 4 second pressure to remove entrapped air between the coverlay film and the electrode substrate. Two mil thick Vacrel 8120 was laminated next to the Vacrel® 8130 surface using the HRL-24 at room temperature, with a roller speed of 1 meter/min. The panel was then vacuum laminated again in the DVL-24 vacuum laminator using a 30 second vacuum dwell, 4 second pressure, to remove entrapped air between the two coverlay films.

The laminated electrode sheet was placed in the Tamarack 152R system and was exposed to ultraviolet light at 365 nm through the photomask for 22 seconds using an exposure intensity of 17 mW/cm^2. The artwork for the capillary chamber was a 1 millimeter by 4 millimeter rectangle centered over the electrode finger array and starting 1 millimeter below the fingers. The exposed coverlay was stripped from the panel to reveal the sensor chamber rectangle using the VLP-20 Circuit Chemistry Equipment) in 1% K_2C0_3, at 140° F., for 75 seconds using a nozzle pressure of 34 psi. The developed laminate structure was rinsed in deionized water, and then cured at 160° C. for 1 hour to thermally crosslink the coverlay material. This completed the construction of the sensor base.

The panel of the base sensors was plasma cleaned to remove residual photoresist and coverlay material from the exposed gold surface of the interdigitated array structure. The panel was placed in a barrel etcher, a Barnstead/IPC model P2100 from Metroline/IPC of Corona, Calif. The panel was first exposed to an oxygen plasma for 1 minute at 800 watts and 1.1 torr pressure to oxidize the panel surface. It was then etched in an oxygen/argon plasma mixture (70/30 vol./vol.) for 3 minutes, at 225 watts and 1.5 torr pressure, and was finally stripped in an argon plasma for 2 minutes, at 150 watts and 2 torr pressure.

The chemical coating was formulated for measurement of d-glucose in a human blood sample. The chemical coating was reactive with the sample in a manner effective to generate an electrical output signal indicative of the level of glucose in the sample. The coating included a mediator, enzymes, and a cofactor. The coating further comprised film forming agents and detergents conferring durability and providing hydrophilicity. The ingredients are listed in Table 1; unless stated otherwise, all concentrations refer to the concentration of a given substance in a wet-coating, prior to the deposition and drying of the coating onto the array.

The chemical coating was formulated from several submixtures of components. A first mixture contained glycerophosphate buffer, from ICN Biomedicals Inc. Aurora, Ohio; Medium Viscosity Alginic acid, from Sigma Chemical Co., St. Louis, Mo.; Natrosol 250M, from Hercules Inc., Wilmington, Del.; and Triton® X-100, from Union Carbide, Danbury Conn. These components were added to a volume of distilled water sufficient to make a 250 gram solution of the buffer/polymer/surfactant (see Table 1). The solution was mixed overnight to allow complete hydration of the Natrosol and Alginic acid. The pH of the completed solution was adjusted to 6.9 to 7.0 with concentrated hydrochloric acid. This solution is known hereinafter as "Solution A."

A second solution prepared was a concentrated enzyme/cofactor matrix. 8.2 milligrams pyrrolo-quinoline-quinone (PQQ), Fluka, Milwaukee, Wis., was added to 25.85 grams of Solution A. The resulting mixture was sonicated until the PQQ was completely in solution. 1.1394 grams of the enzyme, Glucose-De-oxidoreductase (GlucDor), from Roche Molecular Biochemicals, Indianapolis, Ind., was added to the solution. The final mixture was rocked for 2 hours to allow formation of the GlucDor/PQQ holoenzyme. The completed solution will be referred to as "Solution B."

Potassium ferricyanide was added to the composition as follows: 4.4173 grams of potassium ferricyanide, from J. T. Baker, Phillipsburg, N.J., was added to 70.58 grams of Solution A. The resulting solution was mixed until the ferricyanide was completely in solution. The completed solution will be referred to as "Solution C."

The final coating composition was completed by combining 63 grams of Solution C to 25 grams Solution B. This composition was rocked in the dark for 1 hour to thoroughly mix.

TABLE 2

	Formulation per 100 grams of coating		
Component	Concentration/activity	Wet mass (g)	Dry mass/sensor (mg)
Distilled Water		88.487	
Disodium	150 mM	4.359	0.0287
Glycerophosphate	pH 6.98		
Trehalose	1% wt/wt	1.000	0.0066

TABLE 2-continued

Component	Formulation per 100 grams of coating: Concentration/activity	Wet mass (g)	Dry mass/sensor (mg)
Natrosol	0.3% wt/wt	0.300	0.002
Alginic acid Medium viscosity	0.4% wt/wt	0.400	0.0026
Triton X-100	0.025% wt/wt	0.025	0.00016
Pyrrolo-quinoline-Quinone (PQQ)	0.261 mM	0.0082	5.3382 × .10−5
GlucDor Enzyme	2034 u/mg	1.1394	0.0075 15.23 (units)
Potassium Ferricyanide	137 mM	4.2814	0.0281

A preferred method for applying the chemistry matrix to the sensor chamber (IDA) is a discrete dispense of 500 nanoliters of the coating solution into the 1 millimeter×4 millimeter chamber using a microdispensing system such as that sold under the trademark of BioJet Quanti3000™, BioDot Inc., Irvine, Calif. The coating covered both the working and counter electrodes of the IDA. The coating was dried for 1.5 minutes at 45° C. in a horizontal air flow oven, VWR Scientific Products, Chicago Ill.

The hydrophilic top foil was prepared by coating an adhesive mixture (e.g., a mixture of Fastbond™ 30-NF Contact Adhesive and the surfactant Triton™ X-100 (Union Carbide, Danbury Conn.), 93%:7% wt/wt.) to a wet thickness of 25 μm onto 5 mil polyester film such as that sold under the trademark Melinex® "S" (duPont Polyester Films, Wilmington Del.) using a wire bar coater from Thomas Scientific, Swedesboro, N.J. The coated top foil was dried for 2 minutes at 50° C. in a horizontal air flow oven (VWR Scientific Products). The capillary chamber was opened by cutting 1 millimeter in from the front edge of the capillary chamber with a pair of scissors. The dried coated top foil was applied to the sensor, allowing approximately a 0.5 mm space between the back edge of the chamber and the edge of the top foil as an air vent. The top foil was sealed to the sensor surface using a 5 ton press with a heated top platen, at 81° C., 60 psi for 5 seconds. The panel of completed sensors was cut into individual sensors and stored desiccated at 8% RH until tested.

The sensors were evaluated using chronoamperometry electrochemical techniques on test stands such as that sold under the trademark of BAS™ 100W Electrochemical Workstation, Bioanalytical Systems, Inc. West Lafayette, Ind. The preferred electrochemical test stand used in the evaluation of the electrodes was a dedicated test stand for DC chronoamperometric current measurement for assay potentials from ±1 volt.

The sensors may be used to determine the concentration of an analyte, such as glucose, in a fluid sample by performing the following steps:

Set up the test stand parameters:

In accordance with a "drop detect" system, an initial potential difference is established between the working and counter electrodes—300 mV (millivolts)—to start timing of the analysis sequence. Current response to this potential is triggered by contact of the array with a fluid sample.

The initial current response upon application of the test solution to the sensor chamber is generally greater than 0.4 microamps.

The time (delay period) between the threshold trigger and re-application of the 300 mV potential difference (assay potential) is generally 3 seconds.

The assay period, after re-application of the 300 mV potential difference between the working and counter electrodes of the sensor is generally 9 seconds.

In More Detail:

Insert the sensor into the test stand connection. Apply approximately 0.3 uL of a fluid sample to the opening of the capillary chamber. Fluid will flow into the chamber by capillary action covering the chemical coating applied to the working and counter electrodes. The threshold current will be triggered when the sample fluid covers the nearest working and counter electrode fingers. Once triggered, the potential difference will go to open circuit for a 3 seconds, during the delay period.

During the delay period, reaction will occur between the reactants (analyte, enzyme/cofactor, and the oxidized form of the mediator), resulting in reduction of the mediator.

The 300 mV assay potential difference is re-applied between the electrodes after the 3 second delay. This causes electro-oxidation of the reduced mediator at the surface of the working electrode.

The current/time reaction profiles of the assay show a characteristic pseudo-steady-state current/time plateau starting 0.5 to 1.5 seconds after re-application of the 300 mV assay potential to the sensor. Currents at fixed assay period points chosen in this plateau region were proportional to the concentration of analyte in the sample fluid. Assay endpoints were chosen in such a manner give a linear dose response for glucose concentrations from 0 to 600 mg/dL. See FIG. **7**.

EXAMPLE 2

A sensor having an interdigitated array of two electrodes configured for 57 fingers (27 fingers for the working electrode and 28 fingers for the counter electrode) was initially prepared by depositing gold film onto a KALADEX® substrate according the procedure described in Example 1. Each finger of the working electrode and the counter electrode had a width of 50 microns (μm) and was separated from the adjacent finger by a 21 μm gap. The sensor chamber or capillary was fabricated into a coverlay of Vacrel® 8140 material using dry film photolithography. The capillary or chamber had a depth of 0.125 mm and a sample volume of 1.45 μl.

The hydrophilic top foil was prepared by coating an adhesive mixture (e.g., an adhesive mixture of 4.5% TRITON X100®, 4.5% isocyanate (38-8569 from National

Starch and Chemical Co. of Bridgwater, N.J.), and 93% polyurathane (38-8668 also from National Starch and Chemical Co.) to a wet thickness of 25 um onto 5 mil film of Melinex® "S" (duPont Polyester Films, Wilmington Del.) using a wire bar coater from Thomas Scientific, Swedesboro, N.J. The coated top foil was dried for 2 minutes at 50° C. in a horizontal air flow oven (VWR Scientific Products). The capillary chamber was opened by cutting 1 millimeter in from the front edge of the capillary chamber with a pair of scissors. The dried coated top foil was applied to the sensor, allowing approximately a 0.5 mm space between the back edge of the chamber and the edge of the top foil as an air vent. The top foil was sealed to the sensor surface using a 5 ton press with a heated top platen, at 81° C., 60 psi for 5 seconds. The panel of completed sensors was cut into individual sensors and stored desiccated at 8% RH until tested.

The chemical formulations were also prepared as described in Example. 1 (See Table 2.) The chemicals were applied to the sensor chamber at a discrete dispense volume of 1.226 μl into the 2 mm×5.8 mm chamber for each sensor. The resulting sensor had a sample volume of 1.5 μl.

The series of sensors prepared as above described were evaluated by measuring the current across the electrodes produced from a series whole blood test samples spiked with glucose and Hct at varying concentrations. The percentage of Hct and actual glucose concentrations in the test samples are listed below in Table 3.

TABLE 3

Nominal Glucose	Actual Glucose Concentration at various Hematocrit (Hct) levels (mg/dl)				
Conc. mg/dL	20.0% Hct	30% Hct	45% Hct	60% Hct	70% Hct
50	51.7	46.54	38.19	25.78	17.01
100	128.38	121.12	111.64	103.33	92.65
200	201.37	194.21	198.10	188.76	183.44
400	419.79	418.72	414.27	409.85	405.86
600	622.30	612.72	613.76	609.89	593.29

The procedure employed for the evaluation is the same as described in Example 1. The test parameters included a time (delay period) between the threshold trigger and re-application of the 300 mV (dc) potential difference (assay potential) of 3 seconds. Data was collected immediately after the delay period at 4 date points per second for an assay period of about 9 seconds.

The results are illustrated in FIG. **8**. The current/time profiles of the assay were consistent with a characteristic pseudo-steady state current/time plateau at least at 4.5 seconds after dose detect (1.5 sec after reapplication of the 300 mV assay potential to the sensor.) The assay provided linear dose responses for varying glucose concentrations at each of the different Hct levels, with a correlation coefficient (r^2) of greater than 0.979.

EXAMPLE 3

Sensors were prepared according to this method by depositing a gold film onto a flexible substrate as described in Example 1. After the gold was applied to the flexible substrate, a spin on photoresist was applied according to the procedure described in Linder et al. "Flexible Kapton-Based Microsensor Arrays of High Stability for Cardiovascular Applications," *J. Chem. Faraday Trans.* 1993, 89(2), 361–367; Cosofret et al. "Microfabricated Sensor Arrays Sensitive to pH and K+ for Ionic Distribution Measurement in the Beating Heart" *Anal. Chem.* 1995, 67, 1647–1653. The photoresist, (Microposit Shipley 1813 from Shipley of Marlborough Mass.) was spun on to a flexible Kaladex® substrate at 4,000 rpm for 4 seconds. The coated substrate was baked at 90° C. for 15 minutes. The photoresist was exposed through a photomask to uv light at 15.5 mW/cm^2 for 11 seconds. The photomask was patterned to provide a the electrodes with a hook configuration as illustrated in FIG. **9**. The coated substrate was heated to 115° C. for 15 minutes. The photoresist was developed to remove the area exposed to the uv light. The exposed gold was removed with iodide/potassium iodide/water (4:1:40) bath. The photoresist was stripped from the laminated substrate with an acetone/methanol solution. The resulting patterned gold substrate was then dried at 120° C. for 30 minutes. The working electrode had a surface area of 1 mm^2 (1 mm×1 mm); the counter electrode had dimensions of 600 mm length, 2.6 mm+1.8 mm+1.8 mm width). The electrodes were separated by a 200 μm gap.

The resulting substrate was laminated with PYRALUX® PC 1000. The laminated substrate was exposed to uv light at 15.5 mW/cm^2 through a photomask for 11 seconds. The exposed coverlay was developed with a $LiCO_3$ solution and then thermally cured at 160° C. for 60 minutes. The coverlay was fabricated to have a capillary or chamber with a depth of 0.062 mm. The resulting "box hook" sensor had a test sample volume of 0.775 μl.

The sensor was cleaned to remove any residual photoresist and coverlay material. A chemical coating formulation was prepared as described in Example 1. The components and their amounts are listed below in Table 3.

TABLE 4

Component	Formulation per 100 grams of coating: Concentration/ activity	Wet mass (g)	Dry mass/sensor (mg)
Distilled Water		89.51	
Potassium monophosphate	150 mM pH 700	1.2078	0.0121
Potassium diphosphate Buffer		2.7133	0.0271
Trehalose	0.35% wt/wt	0.350	0.0035
Natrosol 250 M	0.060% wt/wt	0.060	0.0006
Polyethylene oxide (100 K)	0.750% wt/wt	0.750	0.0075
Triton X- 100	0.070% wt/wt	0.070	0.0007

TABLE 4-continued

Component	Formulation per 100 grams of coating: Concentration/ activity	Wet mass (g)	Dry mass/sensor (mg)
Pyrrolo-quinoline-Quinone (PQQ)	0.315 mM	0.0104	1.040 × 10−5
GlucDor Enzyme	2624 u/mg (DCIP)	1.1325	0.0113 29.717 units
Potassium Ferricyanide	179.4 mM	5.908	0.0591

A sheet containing several sensors was prepared according to the procedure above described. The sheet was cut to isolate the individual sensors. Lines were drawn on each side of the sensor chamber using a black Sharpie marking pen to define the reaction area for chemistry dispensing. The reagent coating was hand dispensed at a discrete dispense volume of 1.0 μl into the 2.5 mm×5.0 mm chamber for each sensor.

A series of sensors prepared as above described were evaluated by measuring the current generated across the electrodes produced for a series of test samples having differing concentrations of glucose according to the procedure described in Example 1. The test parameters included a time (delay period) between the threshold trigger and re-application of the 300 mV (dc) potential difference (assay potential) of 4 seconds. Data was collected immediately after the delay period at 4 points per second generally for an assay period of 9–12 seconds.

An assay point was chosen from the current/time profiles of the assay at 4.5 sec. after dose detect (0.5 sec after reapplication of the 300 mV assay potential to the sensor. The results are illustrated in FIG. **10**. The assay provided a linear dose response for the different glucose levels, with a correlation coeffecient (r^2) of 0.990.

EXAMPLE 4

A sensor having an interdigitated array of two electrodes and 3 fingers (1 working electrode finger and 2 counter electrode fingers) was initially prepared according the procedure described in Example 3. The electrodes were gold film. Each working electrode finger had a width of 500 μm, and each of the counter electrodes had a width of 500 μm. The electrode array had a gap of 150 μm between the fingers of the working electrode and the adjacent counter electrode. The capillary or chamber was fabricated to have a depth of 0.062 mm and a sample volume of 0.620 μl.

The chemical formulations were also prepared as described in Example. 3 (See Table 3.) The reagent coating was applied to the sensor chamber at a discrete dispense volume of 1.00 μl into the 2 mm×5.0 mm chamber for each sensor.

A series of sensors prepared as above described were evaluated by measuring the current generated across the electrodes produced for a series of test samples having differing concentrations of glucose to the procedure described in Example 1. The test parameters included a time (delay period) between the threshold trigger and re-application of the 300 mV (dc) potential difference (assay potential) of 4 seconds. Data was collected immediately after the delay period at 4 data points per second generally for an assay period of about 9 seconds.

An assay point was chosen from the current/time profiles of the assay at 10 sec. after dose detect (6 sec after reapplication of the 300 mV assay potential to the sensor. The results are illustrated in FIG. **11**. The assay provided a linear dose response for varying glucose concentrations, with a correlation coeffecient (r^2) of 0.965.

EXAMPLE 5

A sensor having an interdigitated array of two electrodes and 5 fingers (2 working electrode fingers and 3 counter electrode fingers) was initially prepared according the procedure described in Example 3. The electrodes were gold film. Each finger of the working electrode had a width of 300 μm, and each finger of the counter electrode had a width of 300 μm. The electrode array had a gap of 300 μm between the working electrode fingers and the counter electrode fingers. The capillary or chamber was fabricated to have a depth of 0.062 mm and a sample volume of 0.620 μl.

The chemical formulations were also prepared as described in Example 3 (See Table 3.) The reagent coating was applied to the sensor chamber at a discrete dispense volume of 1.00 μl into the 2 mm×5.0 mm chamber.

A series of sensors prepared as above described were evaluated by measuring the current generated across the electrodes produced for a series of test samples having differing concentrations of glucose according to the procedure described in Example 1. The test parameters included a time (delay period) between the threshold trigger and re-application of the 300 mV (de) potential difference (assay potential) of 4 seconds. Data was collected immediately after the delay period at 4 data points per second generally for an assay period of about 9 seconds.

An assay point was chosen from the current/time profiles of the assay at 10 sec. after dose detect (6 sec after reapplication of the 300 mV assay potential to the sensor. The results are illustrated in FIG. **12**. The assay provided a linear dose response for the different glucose concentrations, with a correlation coeffecient (r^2) of 0.973.

EXAMPLE 6

A sensor having an interdigitated array of two electrodes and 29 fingers (14 working electrode fingers and 15 counter electrodes fingers) was initially prepared according the procedure described in Example 2. The electrodes were gold film. Each finger of the working electrode had a width of 50 μm, and each finger of the counter electrode had a width of 50 μm. The electrode array had a gap of 25 μm between the fingers of working electrode and the adjacent finger of the counter electrode. The capillary or chamber was fabricated to have a depth of 0.127 mm and a sample volume of 1.02 μl.

The coverlay material was prepared by laminating a two layers of PYRALUX® PC 1000.

The chemical formulations were also prepared as described in Example. 2 (See Table 3.) The chemicals were applied to the sensor chamber at a discrete dispense volume of 1.00 μl into the 2 mm×5.0 mm chamber.

A series of sensors prepared as above described were evaluated by measuring the current generated across the electrodes produced for a series of test samples having differing concentrations of glucose at various Hct levels according to the procedure described in Example 1. The actual glucose concentration of each sample was determined as listed in Table 4.

TABLE 5

Nominal Glucose Concentration	Actual Glucose Conc. At various Hematocrit (Hct) levels (mg/dl)				
mg/dL	0.0%	20%	40%	55%	70%
0	0.0	0.0	0.0	0.0	0.0
50	48.5	47.2	45.15	40.70	42.85
100	94.75	94.75	92.25	91.30	81.60
300	290.75	291.5	289.2	276.85	294.20
600	575.05	569.15	574.95	548.55	555.0

The test parameters included a time (delay period) between the threshold trigger and re-application of the 300 mV (dc) potential difference (assay potential) of 2 seconds. Data was collected immediately after the delay period at 20 data points per second generally for an assay period of about 9 seconds.

The results are illustrated in FIG. **13**. An assay point was selected from the current/time profiles of the assay at 2.1 seconds after dose detect (0.1 seconds after reapplication of the 300 mV assay potential to the sensor. The assay provided a linear dose response for varying glucose concentrations at different Hct levels, with a correlation coeffecient (r^2) of greater than 0.988 (See FIG. **14**.)

What is claimed is:

1. A method of determining the concentration of glucose in a blood sample, comprising:

providing a disposable biosensor test strip including a capillary chamber having a depth suitable for capillary flow of blood and holding a volume of between about 0.1 μl and about 1.0 μl of the blood sample, a working electrode and a counter or reference electrode disposed within the capillary chamber, and a reagent proximal to or in contact with at least the working electrode, the reagent including an enzyme and a mediator, the reagent reacting with glucose to produce an electroactive reaction product;

applying a blood sample containing glucose into the capillary chamber, the capillary chamber directing capillary flow of the blood sample into contact with the reagent to cause the blood sample to at least partially solubilize or hydrate the reagent;

detecting the blood sample in the capillary chamber;

following said detecting, applying or controlling the voltage or current across the working and counter or reference electrodes;

electrooxidizing or electroreducing the electroactive reaction product at the working electrode; and

within 10 seconds after said detecting, determining and providing a readout of the glucose concentration in the blood sample, said determining comprising correlating the electrooxidized or electroreduced electroactive reaction product to the concentration of glucose in the blood sample.

2. The method of claim **1** in which the capillary chamber holds a volume of between about 0.4 μl and about 1.0 μl.

3. The method of claim **1** which includes determining and providing a readout of the glucose concentration within about 8 seconds after said detecting.

4. The method of claim **3** which includes determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

5. The method of claim **1** in which said determining and providing a readout of the concentration comprises determining the concentration from about 0.5 to about 2 seconds after said applying or controlling.

6. The method of claim **1** in which said applying or controlling comprises applying a DC voltage of 100–500 mV between the working and counter or reference electrodes, said determining the concentration comprising measuring the amount of the electrooxidized or electroreduced electroactive reaction product and correlating the measurement to the amount of glucose in the sample.

7. The method of claim **6** in which the DC voltage is about 300 mV.

8. The method of claim **6** in which said measuring the amount of the electrooxidized or electroreduced electroactive reaction product comprises measuring the current and correlating the measured current to the concentration of the glucose.

9. The method of claim **6** in which said measuring the amount of the electrooxidized or electroreduced electroactive reaction product comprises measuring the amount from about 0.5 to about 2 seconds after said applying or controlling.

10. The method of claim **1** in which said providing comprises providing the reagent in a sufficiently small amount as to be solubilized or hydrated in a time sufficient to allow said determining and providing a readout of the glucose concentration in the sample within 10 seconds after said detecting.

11. The method of claim **1** in which the working and counter or reference electrodes are coplanar.

12. The method of claim **1** in which the test strip includes a vent in communication with the capillary chamber to facilitate flow of the sample into the capillary chamber.

13. The method of claim **1** in which said determining comprises determining the glucose concentration over the range of 0–600 mg/dL.

14. The method of claim **1** in which said detecting comprises applying a potential difference between electrodes within the capillary chamber.

15. The method of claim **14** in which said detecting comprises applying a potential difference between the working and counter or reference electrodes prior to and separate from said applying or controlling the voltage or current across the working and counter or reference electrodes.

16. The method of claim **1** in which said capillary chamber has a depth of 25–200 um.

17. The method of claim **1** which includes automatically operating the test strip and timing the reaction and analysis of the blood sample to detect the blood sample in the capillary chamber, to electrooxidize the electroactive reaction product, and to determine and provide a readout of the glucose concentration within 10 seconds of said detecting.

18. The method of claim **1** in which the test strip comprises a counter electrode, and in which the reagent is located proximal to or in contact with the working and counter electrodes.

19. The method of claim **1** in which the capillary chamber holds a volume of about 600 nL.

20. The method of claim **19** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

21. The method of claim **1** in which the capillary chamber holds a volume of between 0.25 μl and 0.4 μl.

22. The method of claim **21** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

23. The method of claim **21** in which the capillary chamber holds a volume of about 400 nL.

24. The method of claim **23** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

25. The method of claim **21** in which the capillary chamber holds a volume of about 300 nL.

26. The method of claim **25** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

27. The method of claim **1** comprising determining and providing a readout of the glucose concentration about 3.5 to about 8 seconds after said detecting.

28. The method of claim **27** comprising determining and providing a readout of the glucose concentration within about 4 seconds of said detecting.

29. The method of claim **27** comprising determining and providing a readout of the glucose concentration about 5 seconds after said detecting.

30. The method of claim **27** comprising determining and providing a readout of the glucose concentration about 4 seconds after said detecting.

31. A method of determining the concentration of glucose in a blood sample, comprising:

providing a disposable biosensor test strip including a capillary chamber having a depth suitable for capillary flow of blood and holding a volume of between about 0.1 μl and about 1.0 μl of the blood sample, a working electrode and a counter or reference electrode disposed within the capillary chamber, and a reagent proximal to or in contact with at least the working electrode, the reagent including an enzyme and a mediator, the reagent reacting with glucose to produce an electroactive reaction product;

applying a blood sample containing glucose into the capillary chamber, the capillary chamber directing capillary flow of the blood sample into contact with the reagent to cause the blood sample to at least partially solubilize or hydrate the reagent;

detecting the blood sample in the capillary chamber;

following said detecting, applying or controlling the voltage or current across the working and counter or reference electrodes;

electrooxidizing the electroactive reaction product at the working electrode; and

within 10 seconds after said detecting, determining and providing a readout of the glucose concentration in the blood sample, said determining comprising correlating the electrooxidized electroactive reaction product to the concentration of glucose in the blood sample.

32. The method of claim **31** in which the capillary chamber holds a volume of about 600 nL.

33. The method of claim **32** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

34. The method of claim **31** in which the capillary chamber holds a volume of between 0.25 μl and 0.4 μl.

35. The method of claim **34** comprising detennining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

36. The method of claim **34** in which the capillary chamber holds a volume of about 400 nL.

37. The method of claim **36** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

38. The method of claim **34** in which the capillary chamber holds a volume of about 300 nL.

39. The method of claim **38** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

40. The method of claim **31** comprising determining and providing a readout of the glucose concentration within about 8 seconds of said detecting.

41. The method of claim **40** comprising determining and providing a readout of the glucose concentration about 3.5 to about 8 seconds after said detecting.

42. The method of claim **41** comprising determining and providing a readout of the glucose concentration within about 5 seconds of said detecting.

43. The method of claim **42** comprising determining and providing a readout of the glucose concentration within about 4 seconds of said detecting.

44. The method of claim **41** comprising determining and providing a readout of the glucose concentration about 5 seconds after said detecting.

45. The method of claim **41** comprising determining and providing a readout of the glucose concentration about 4 seconds after said detecting.

46. The method of claim **31** in which said reagent is dry, and the capillary chamber directs capillary flow of the blood sample into contact with the dry reagent to cause the blood sample to at least partially solubilize or hydrate the dry reagent.

47. The method of claim **46** in which the dry reagent comprises a reagent that is applied wet and dried of solvent.

48. The method of claim **31** in which the capillary chamber has a depth of 25-200 μm.

49. The method of claim **31** in which said providing comprises providing the reagent in a sufficiently small amount as to be solubilized or hydrated in a time sufficiently fast to allow said determining and providing a readout of the glucose concentration in the blood sample within 10 seconds of said detecting.

50. The method of claim **49** in which the mediator reacts sufficiently rapidly as to allow determining and providing a readout of the glucose concentration in the blood sample within 10 seconds of said detecting.

51. The method of claim **50** in which the mediator is readily-reversible.

52. The method of claim **31** which includes automatically operating the test strip and timing the reaction and analysis of the blood sample to detect the blood sample in the capillary chamber, to electrooxidize the electroactive reaction product, and to determine and provide a readout of the glucose concentration within 10 seconds of said detecting.

53. The method of claim **52** in which said automatically operating comprises connecting the test strip to an external testing apparatus prior to said detecting, the testing apparatus automatically detecting the blood sample in the capillary chamber, electrooxidizing the electroactive reaction product, determining the glucose concentration, and providing a readout of the glucose concentration within 10 seconds of said detecting.

54. The method of claim 31 in which the working electrode and the counter or reference electrode are coplanar.

55. The method of claim 31 in which said detecting comprises applying a drop-detect potential across the working and counter or reference electrodes.

56. The method of claim 55 in which said detecting comprises applying a drop-detect potential across the working and counter or reference electrodes prior to and separate from said determining.

57. The method of claim 56 in which said detecting comprises applying a drop-detect potential across the working and counter or reference electrodes, recognizing a rise in current as an indication that the blood sample has been applied into the capillary chamber, and discontinuing the drop-detect potential.

58. The method of claim 56 which includes reapplying a potential across the working and counter or reference electrodes, after a delay period during which no potential is applied, to electrooxidize the electroactive reaction product at the working electrode.

59. The method of claim 31 in which said providing comprises providing a test strip including a bottom substrate, a spacing layer, and a top substrate, the spacing layer having an opening corresponding to the capillary chamber, the spacing layer substantially defining the depth of the capillary chamber.

60. The method of claim 31 in which the reagent comprises a mediator, and in which said reacting produces a reduced form of the mediator.

61. The method of claim 31 in which said determining comprises measuring the amount of the electro oxidized or electroreduced electro active reaction product and correlating the amount to the concentration of glucose in the blood sample.

62. The method of claim 31 in which the test strip comprises a counter electrode, and in which the reagent is located proximal to or in contact with the working and counter electrodes.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,276,146 C1
APPLICATION NO. : 90/010079
DATED : April 7, 2009
INVENTOR(S) : Christopher D. Wilsey

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the cover page, Item (73) Assignee, replace --Corange International Limited, Hamilton (BM)-- with "Roche Diagnostics Operations, Inc., Indianapolis, IN (US); Roche Operations Ltd., Hamilton (BM)"

Col. 2, line 20, replace --Vaccrel®-- with "Vacrel®"

Col. 2, line 23, replace --145 µl-- with "1.45 µl"

Col. 2, line 27, replace --X100 200-- with "X100®"

Col. 2, line 50, replace --Actual-- with "actual"

Col. 4, line 34, replace --appling-- with "applying"

Col. 4, line 38, replace --claime-- with "claim"

Signed and Sealed this

Second Day of March, 2010

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

US007276146C1

(12) EX PARTE REEXAMINATION CERTIFICATE (6758th)

United States Patent

Wilsey

(10) Number: US 7,276,146 C1
(45) Certificate Issued: *Apr. 7, 2009

(54) **ELECTRODES, METHODS, APPARATUSES COMPRISING MICRO-ELECTRODE ARRAYS**

(75) Inventor: **Christopher D. Wilsey**, Carmel, IN (US)

(73) Assignee: **Corange International Limited**, Hamilton (BM)

Reexamination Request:
No. 90/010,079, Dec. 14, 2007

Reexamination Certificate for:
Patent No.: **7,276,146**
Issued: **Oct. 2, 2007**
Appl. No.: **10/264,891**
Filed: **Oct. 4, 2002**

(*) Notice: This patent is subject to a terminal disclaimer.

Related U.S. Application Data

(60) Provisional application No. 60/332,411, filed on Nov. 16, 2001.

(51) **Int. Cl.**
G01N 27/327 (2006.01)

(52) **U.S. Cl.** **205/792**; 205/777.5; 204/403.04

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,153,069 A 11/2000 Pottgen et al.

FOREIGN PATENT DOCUMENTS

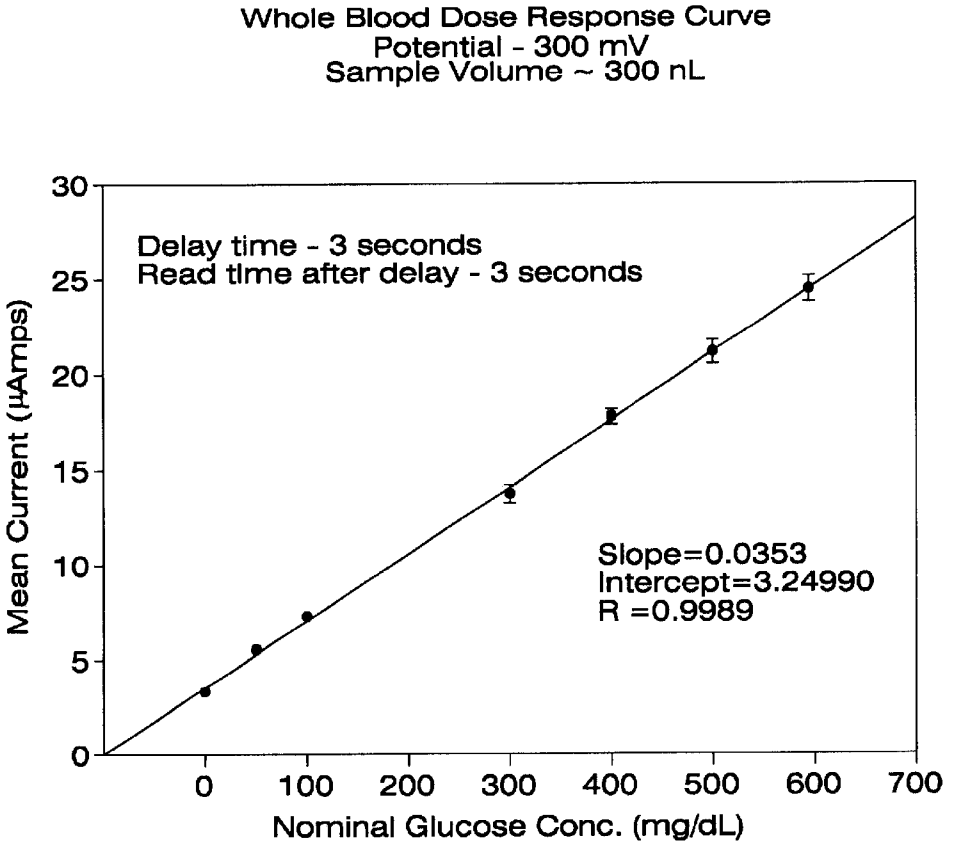
WO WO 00/20626 A1 4/2000

Primary Examiner—Gary L Kunz

(57) **ABSTRACT**

Described are micro-arrays of electrodes disposed proximal to a flexible substrate, electronic components and sensors comprising such arrays, and methods of use for such arrays.

AMENDED



AMENDED

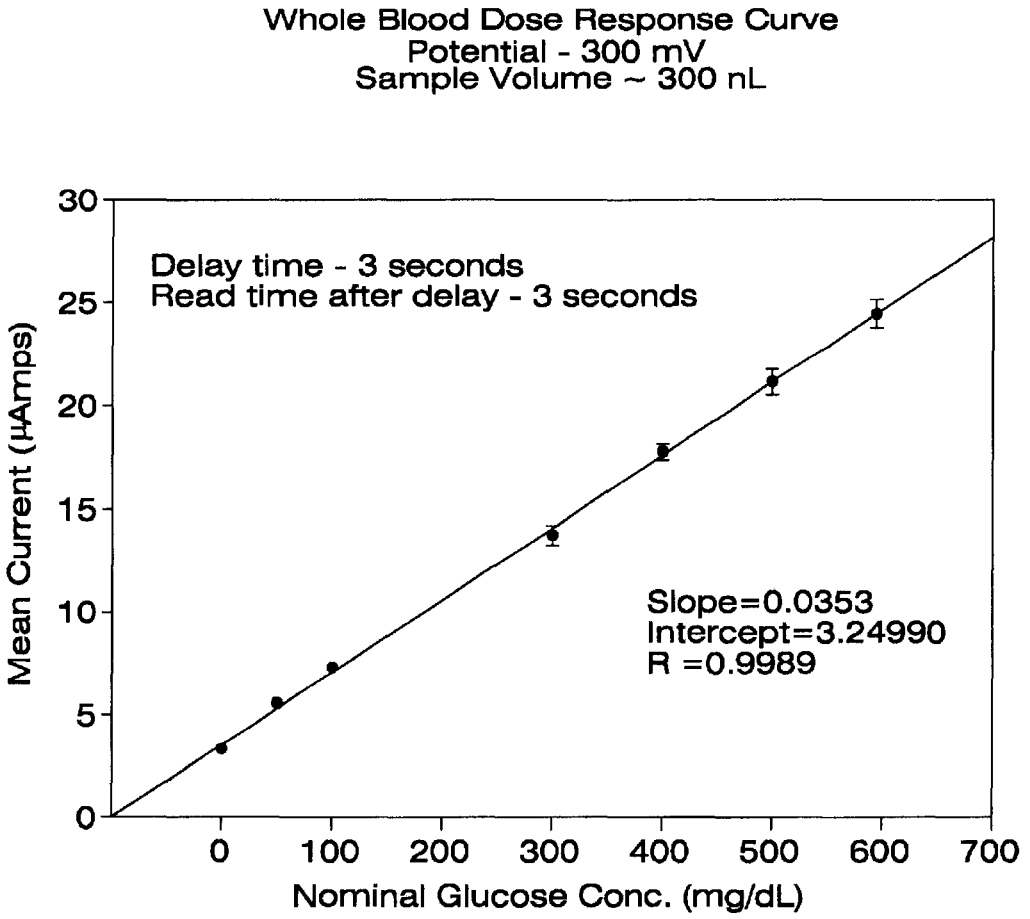


Fig. 7

AMENDED

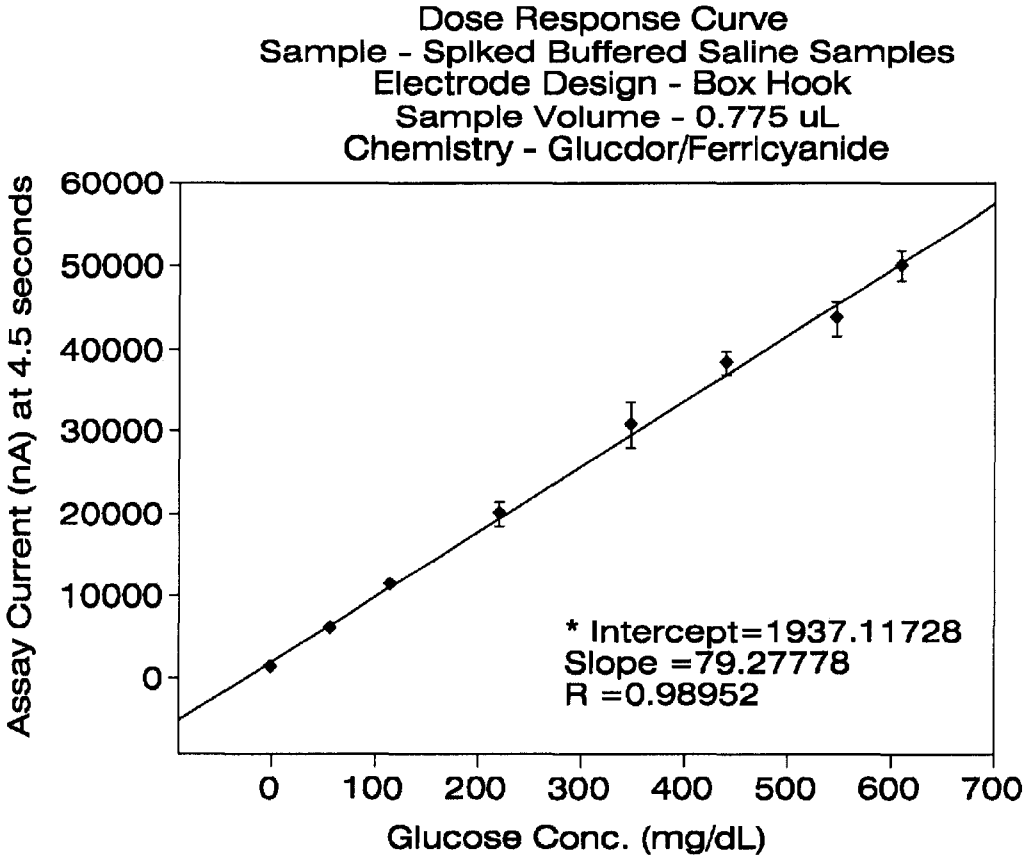


Fig. 10

AMENDED

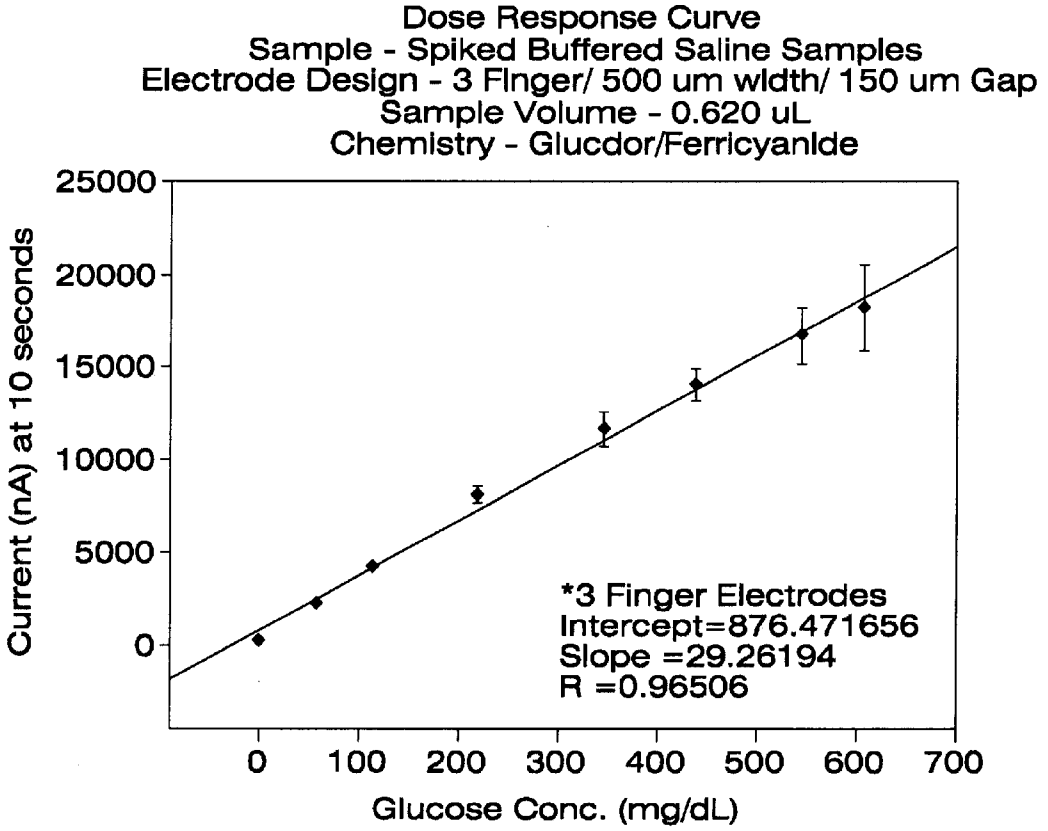


Fig. 11

AMENDED

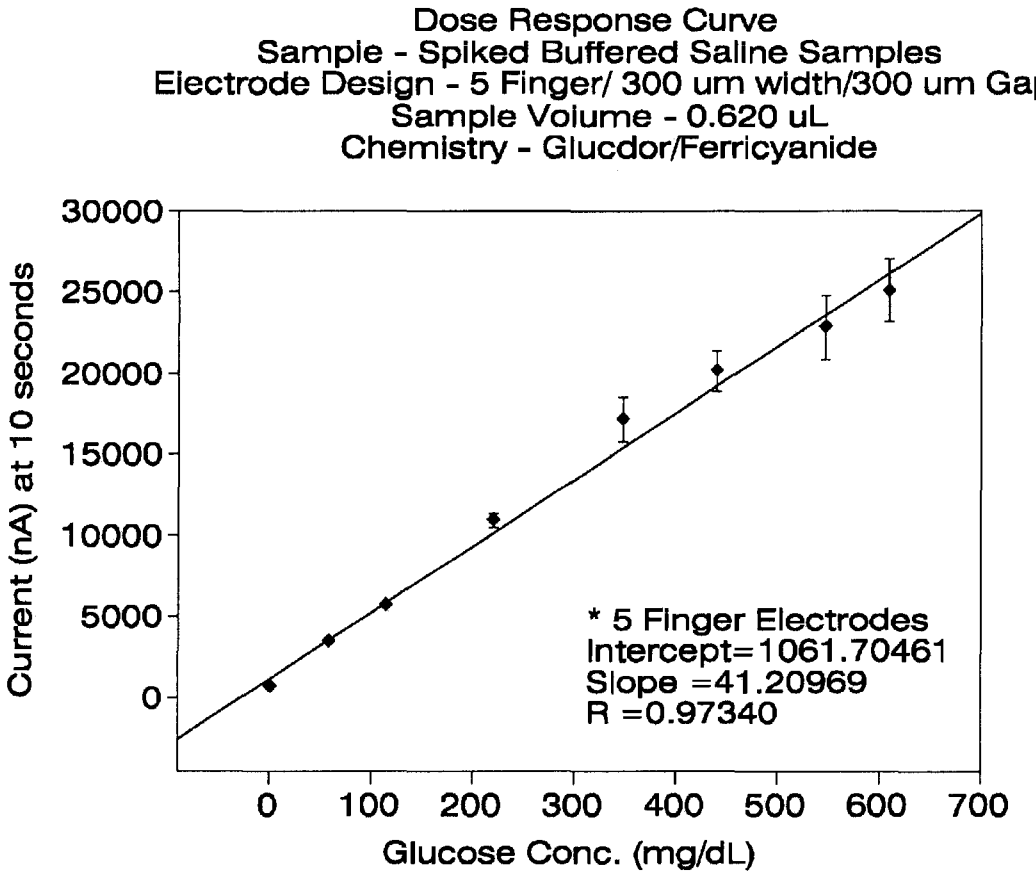


Fig. 12

EX PARTE REEXAMINATION CERTIFICATE ISSUED UNDER 35 U.S.C. 307

THE PATENT IS HEREBY AMENDED AS INDICATED BELOW.

Matter enclosed in heavy brackets [ ] appeared in the patent, but has been deleted and is no longer a part of the patent; matter printed in italics indicates additions made to the patent.

ONLY THOSE PARAGRAPHS OF THE SPECIFICATION AFFECTED BY AMENDMENT ARE PRINTED HEREIN.

Column 18, line 54 to column 19, line 6:

Methods useful to place a microchannel or capillary onto the array include methods of mechanical lamination and mechanical removal of material to form a channel or capillary. One method would include a first step of mechanically "punching" (e.g., die punching) the coverlay material to cut away one or multiple portions of the material in the form of wells or channels, and then laminating the material to one or a number of sensors such that the channel is present over the array. Another method includes those types of methods generally referred to as "kiss die cutting" or "kiss cutting," which may be used to cut a well or channel in a coverlay layer, and then the coverlay material may be laminated onto the **[**substrate**]** *substrate* with the well or channel over the array. **[**One method of producing wells in a coverlay material is described, for example, in U.S. patent application Ser. No. 60/332,192, entitled "Methods to Fabricate Biomedical Devices with Wells and Micro-Environments and Associated Products," filed on even date herewith, and having attorney docket No. 5051-552PR, the disclosure which is inccorporated herein by reference.**]**

Column 19, lines 7–23:

A different example that includes a die punching method is as follows. A spacer foil was prepared by coating an adhesive, FastbondTM 30-NF Contact Adhesive to a wet thickness of 25 μm onto a 5 mil polyester film such as that sold under the trademark Melinex® S (DuPont Polyester films, Wilmington Del.) using a wire bar coater from Thomas Scientific of Swedesboro, N.J. The coated top foil was dried for 2 minutes at 50**[**C.**]** ° *C.* in a horizontal air flow oven. The dried adhesive on the sheet was covered with either silicon or teflon release liner. Capillary channels and electrode contact well patterns were kiss cut into the sheet using an Aristomat 1310 **[**ditigal**]** *digital* die cutting system (Aristo Graphic Systeme GmbH & Co., **[**Hambrug**]** *Hamburg* Germany). The spacer sheet can then be registered and laminated to an ablated sheet of sensor traces, as described above. Channels and electrode contact wells can also be produced using die punching processes in a similar fashion.

Column 20, lines 42–50:

The interdigitated array and connectors can be fabricated using batch photolithography processes common to the flex circuit industry. Electrodes with combinations of finger width and spacing between fingers in the range of 21 to 50 **[**um**]** *μm* were easily fabricated using these processes. A preferred configuration of the array was 21 total fingers (10 working electrode fingers and 11 counter electrode fingers), with finger dimensions of 25 microns (width) by 1 millimeter (length), with 21 micron spacing between the fingers.

Column 24, lines 26–34:

Insert the sensor into the test stand connection. Apply approximately 0.3 μL of a fluid sample to the opening of the capillary chamber. Fluid will flow into the chamber by capillary acttion covering the chemical coating applied to the working and counter electrodes. The threshold current will be triggered when the sample fluid covers the nearest working and counter electrode fingers. Once triggered, the potential difference will go to open circuit for **[**a**]** 3 seconds, during the delay period.

Column 24, lines 53–64:

A sensor having an interdigitated array of two electrodes configured for 57 fingers (27 fingers for the working electrode and 28 fingers for the counter electrode) was initially prepared by depositing gold film onto a KALADEX® substrate according *to* the procedure described in Example 1. Each finger of the working electrode and the counter electrode had a width of 50 microns (μm) and was separated from the adjacent finger by a 21 μm gap. The sensor chamber or capillary was fabricated into a coverlay of Vaccrel® 8140 material using dry film photolithography. The capillary or chamber had a depth of 0.125 mm and a sample volume of 145 μl.

Column 24, line 65 to column 25, line 17:

The hydrophilic top foil was prepared by coating an adhesive mixture (e.g., an adhesive mixture of 4.5% TRITON X100 200 , 4.5% isocyanate (38-8569 from National Starch and Chemical Co. of Bridgewater, N.J.), and 93% polyurathane (38-8668 also from National Starch and Chemical Co.) to a wet thickness of 25 **[**um**]** *μm* onto 5 mil film of Melinex® "S" (duPont Polyester Films, Wilmington Del.) using a wire bar coater from Thomas Scientific, Swedesboro, N.J. The coated top foil was dried for 2 minutes at 50° C. in a horizontal air flow oven (VWR Scientific Products). The capillary chamber was opened by cutting 1 millimeter in from the front edge of the capillary chamber with a pair of scissors. The dried coated top foil was applied to the sensor, allowing approximately a 0.5 mm space between the back edge of the chamber and the edge of the top foil as an air vent. The top foil was sealed to the sensor surface using a 5 ton press with a heated top platen, at 81° C., 60 psi for 5 seconds. The panel of completed sensors was cut into individual sensors and stored desiccated at 8% RH unil tested.

Column 25, lines 23–28:

The series of sensors prepared as above described were evaluated by measuring the current across the electrodes produced from a series *of* whole blood test samples spiked with glucose and Hct at varying concentrations. The percentage of Hct and Actual glucose concentrations in the test samples are listed below in Table 3.

Column 25, lines 42–48:

The procedure employed for the evaluation is the same as described in Example 1. The test parameters included a time (delay period) between the threshold trigger and reapplication of the 300 mV (dc) potential difference (assay potential) of 3 seconds. Data was collected immediately after the delay period at 4 **[**date**]** *data* points per second for an assay period of about 9 seconds.

Column 26, lines 10–37:

Sensors were prepared according to this method by depositing a gold film onto a flexible substrate as described in Example 1. After the gold was applied to the flexible substrate, a spin on photoresist was applied according to the procedure described in Linder et al. "Flexible Kapton-Based Microsensor Arrays of High Stability for Cardiovascular Applications," *J. Chem. Faraday Trans.* 1993, 89(2),

361–367; Cosofret et al. "Microfabricated Sensor Arrays Sensitive to pH and K+ for Ionic Distribution Measurement in the Beating Heart" *Anal. Chem.* 1995, 67, 1647–1653. The photoresist, (Microposit Shipley 1813 from Shipley of Marlborough Mass.) was spun on to a flexible Kaladex® substrate at 4,000 rpm for 4 seconds. The coated substrate was baked at 90° C. for 15 minutes. The photoresist was exposed through a photomask to uv light at 15.5 mW/cm^2 for 11 seconds. The photomask was patterned to provide **[**a**]** the electrodes with a hook configuration as illustrated in FIG. **9**. Tthe coated substrate was heated to 115° C. for 15 minutes. The photoresist was developed to remove the area exposed to the uv light. The exposed gold was removed with iodide/potassium iodide/water (4:1:40) bath. The photoresist was stripped from the laminated substrate with an acetone/methanol solution. The resulting patterned gold substrate was then dried at 120° C. for 30 minutes. The working electrode had a surface area of 1 mm^2 (1 mm×1 mm); the counter electrode had dimensions of 600 mm length, 2.6 mm+1.8 +1.8 mm width). The electrodes were separated by a 200 μm gap.

Column 26, line 51 to Column 27, line 13:

TABLE 4

Formulation per 100 grams of coating

Component	Concentration/ activity	Wet mass (g)	Dry mass/ sensor (mg)
Distilled Water		89.51	
Potassium monophosphate		1.2078 2.7133	0.0121 0.271
Potassium diphosphate Buffer	150 mM pH [700] *7.00*		
Trehalose	0.35% wt/wt	0.350	0.0035
Natrosol 250 M	0.060% wt/wt	0.060	0.0006
Polyethylene oxide (100 K)	0.750% wt/wt	0.750	0.0075
Triton X-100	0.070% wt/wt	0.070	0.0007
Pyrrolo-quinoline Quinone (PQQ)	0.315 mM	0.0104	1.040 × 10^{-5}
GlucDor Enzyme	2624 u/mg (DCIP)	1.1325	0.0113 29.717 units
Potassium Ferricyanide	179.4 mM	5.908	0.0591

Column 27, lines 41–50:

A sensor having an interdigitated array of two electrodes and 3 fingers (1 working electrode finger and 2 counter electrode fingers) was initially prepared according *to* the procedure described in Example 3. The electrodes were gold film. Each working electrode finger had a width of 500 μm, and each of the counter electrodes had a width of 500 μm. The electrode array had a gap of 150 μm between the fingers of the working electrode and the adjacent counter electrode. The capillary or chamber was fabricated to have a depth of 0.062 mm and a sample volume of 0.620 μl.

Column 28, lines 36–45:

A series of sensors prepared as above described were evaluated by measuring the current generated across the electrodes produced for a series of test samples having differing concentrations of glucose according to the procedure described in Example 1. The test parameters included a time (delay period) between the threshold trigger and re-application of the 300 mV **[**(de)**]** (*dc*) potential difference (assay potential) of 4 seconds. Data was collected immediately after the delay period at 4 data points per second generally for an assay period of about 9 seconds.

THE DRAWING FIGURES HAVE BEEN CHANGED AS FOLLOWS:

FIG. **7**: "uAmps" changed to "μAmps".

FIGS. **10**, **11**, and **12**: on Y-axis—changed (uAmps) to (nA).

AS A RESULT OF REEXAMINATION, IT HAS BEEN DETERMINED THAT:

The patentability of claims **1**–**4**, **6**–**12**, **14**, **15**, **17**–**34**, **36**–**60** and **62** is confirmed.

Claims **5**, **13**, **16**, **35** and **61** are determined to be patentable as amended.

:

5. The method of claim **1** **[**in which said**]** *comprising* determining and providing a readout of the concentration **[**comprises determining the concentration**]** from about 0.5 to about 2 seconds after said appling or controlling.

13. The method of claim **1** **[**in which said**]** *comprising determining* **[**comprises determining**]** the glucose concentration over the range of 0–600 mg/dL.

16. The method of claime **1** in which said capillary chamber has a depth of 25–200 **[**um**]** *μm*.

35. The method of claim **34** comprising **[**detennining**]** *determining* and providing a readout of the glucose concentration within about 5 seconds after said detecting.

61. The method of claim **31** in which said determining comprises measuring the amount of the **[**electro oxidized**]** *electrooxidized* or electroreduced **[**electro active**]** *electroactive* reaction product and correlating the amount to the concentration of glucose in the blood sample.

* * * * *

US007276147B2

(12) **United States Patent**
Wilsey

(10) **Patent No.: US 7,276,147 B2**
(45) **Date of Patent: *Oct. 2, 2007**

(54) **METHOD FOR DETERMINING THE CONCENTRATION OF AN ANALYTE IN A LIQUID SAMPLE USING SMALL VOLUME SAMPLES AND FAST TEST TIMES**

(75) Inventor: **Christopher D. Wilsey**, Carmel, IN (US)

(73) Assignees: **Roche Diagnostics Operations, Inc.**, Indianapolis, IN (US); **Corange International Limited**, Hamilton (BM)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/382,322**

(22) Filed: **Mar. 5, 2003**

(65) **Prior Publication Data**

US 2004/0031682 A1 Feb. 19, 2004

Related U.S. Application Data

(63) Continuation of application No. 10/264,785, filed on Oct. 4, 2002.

(60) Provisional application No. 60/332,411, filed on Nov. 16, 2001.

(51) **Int. Cl.**
G01N 27/327 (2006.01)

(52) **U.S. Cl.** **205/792**; 205/777.5; 204/403.04

(58) **Field of Classification Search** 204/403.01–403.15, 416–418; 205/777.5, 205/778, 792

See application file for complete search history.

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Primary Examiner—Alex Noguerola
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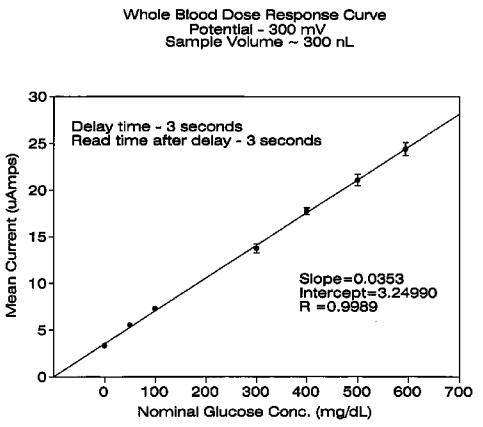
(57) **ABSTRACT**

Analytes in a liquid sample are determined by methods utilizing sample volumes of less than about 1.5 µl and test times within ten seconds. The methods are preferably performed using small test strips including a sample receiving chamber filled with the sample by capillary action.

69 Claims, 6 Drawing Sheets



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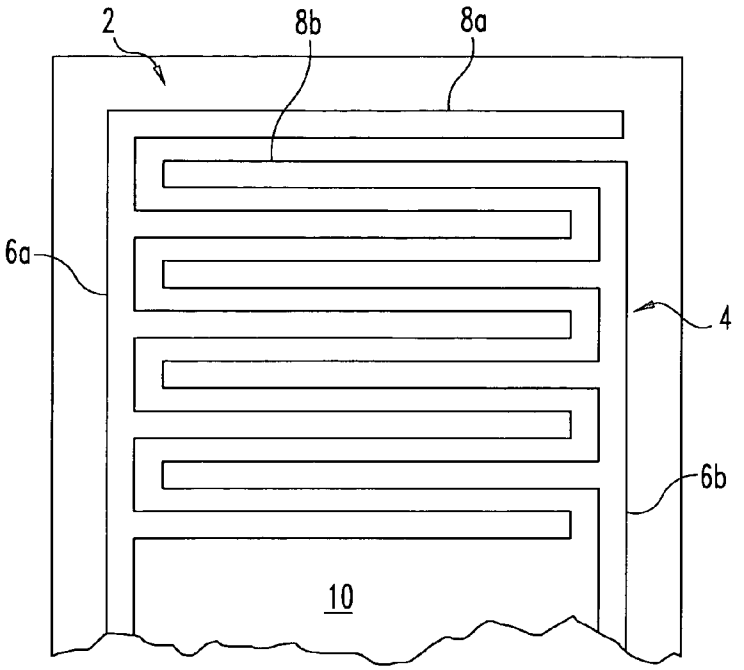

Fig. 1

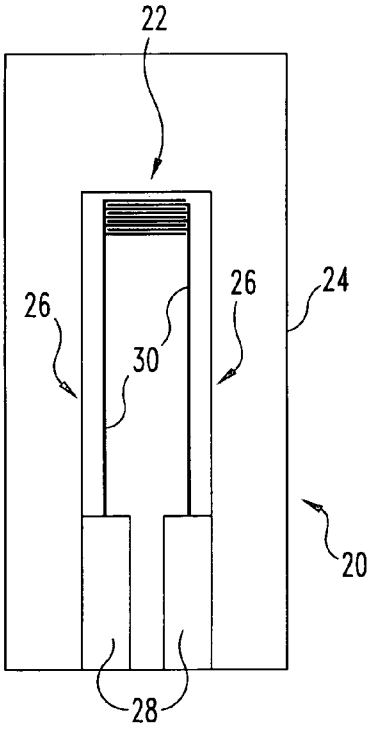

Fig. 2

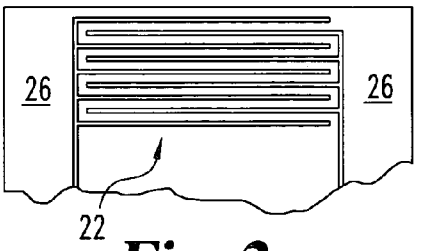

Fig. 2a

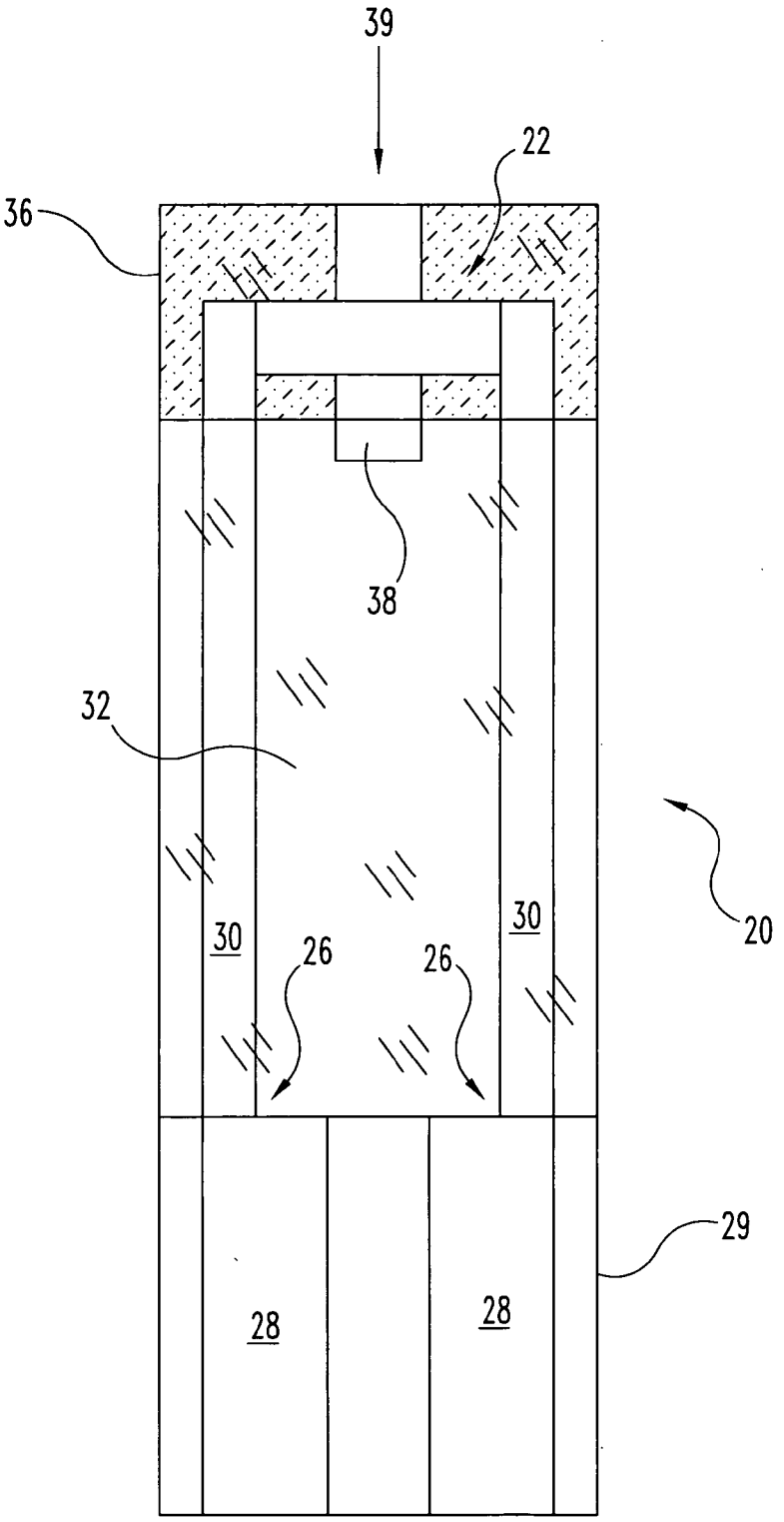

Fig. 3

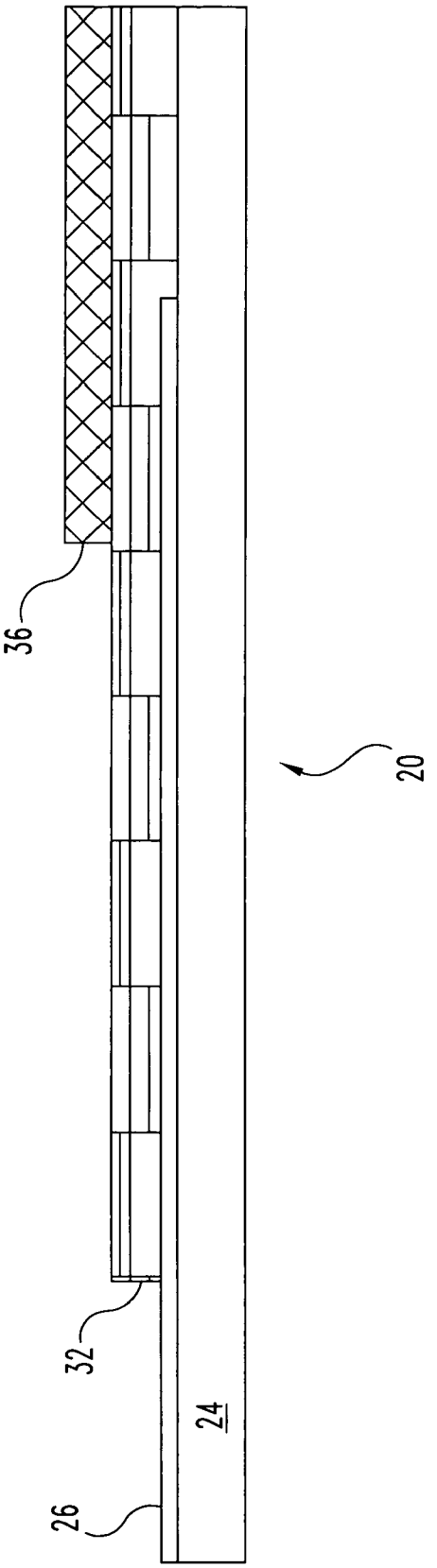

Fig. 4

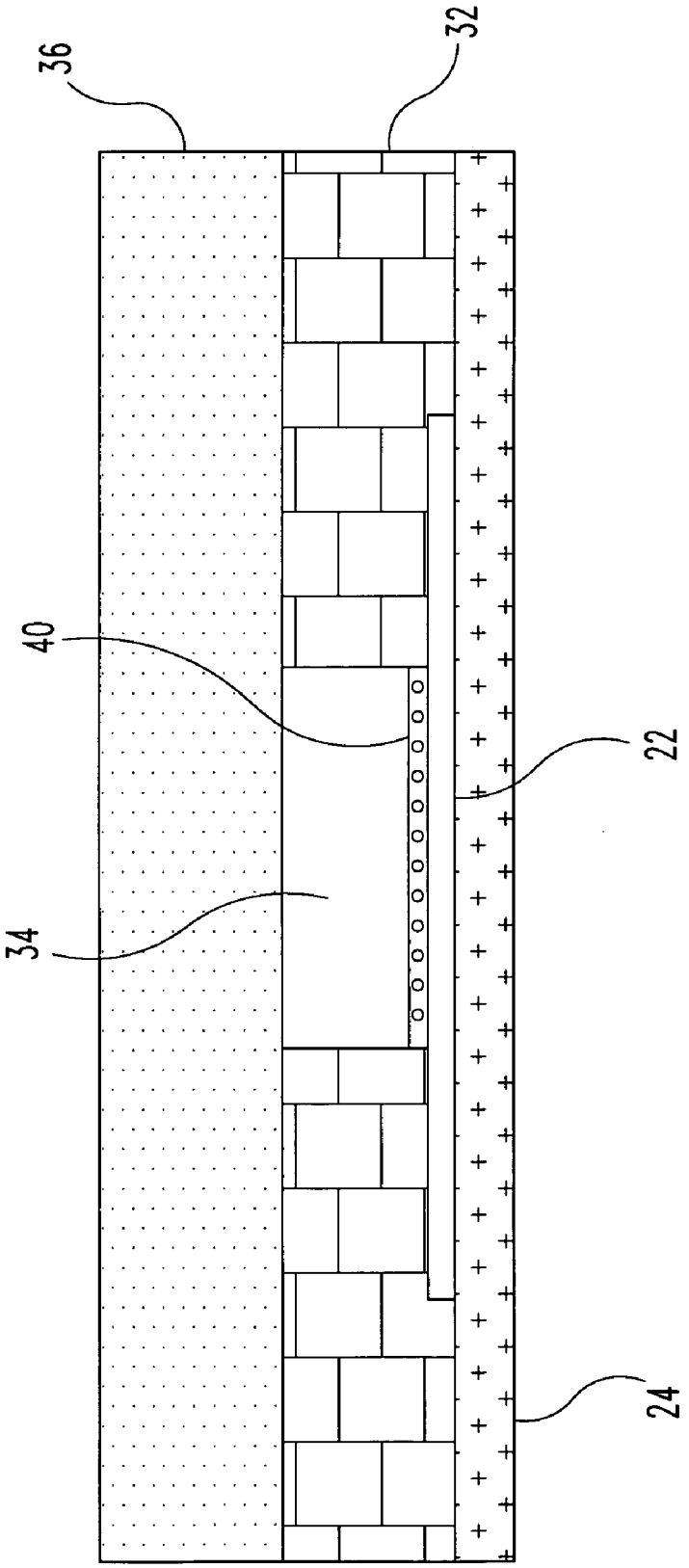

Fig. 5

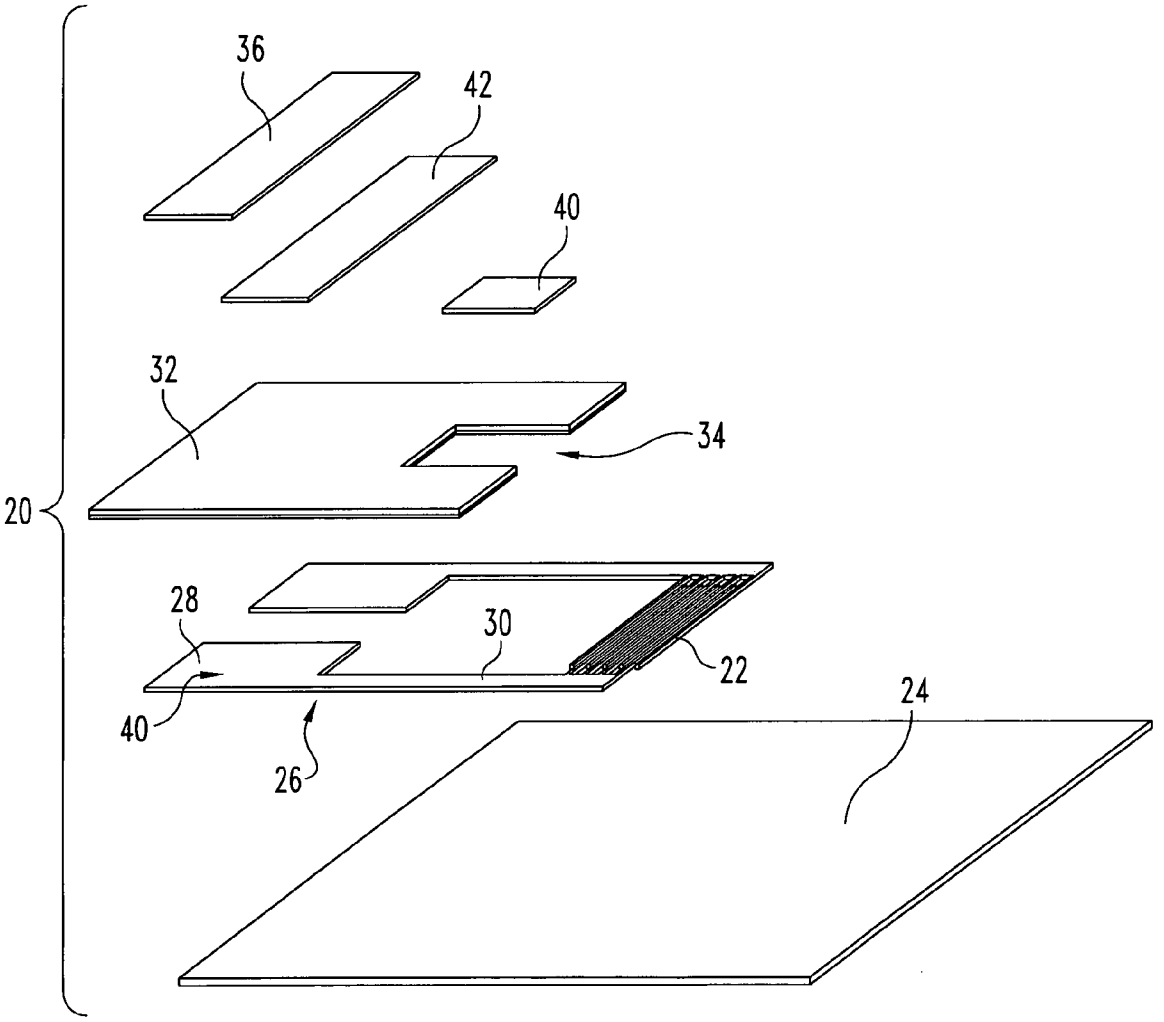

Fig. 6

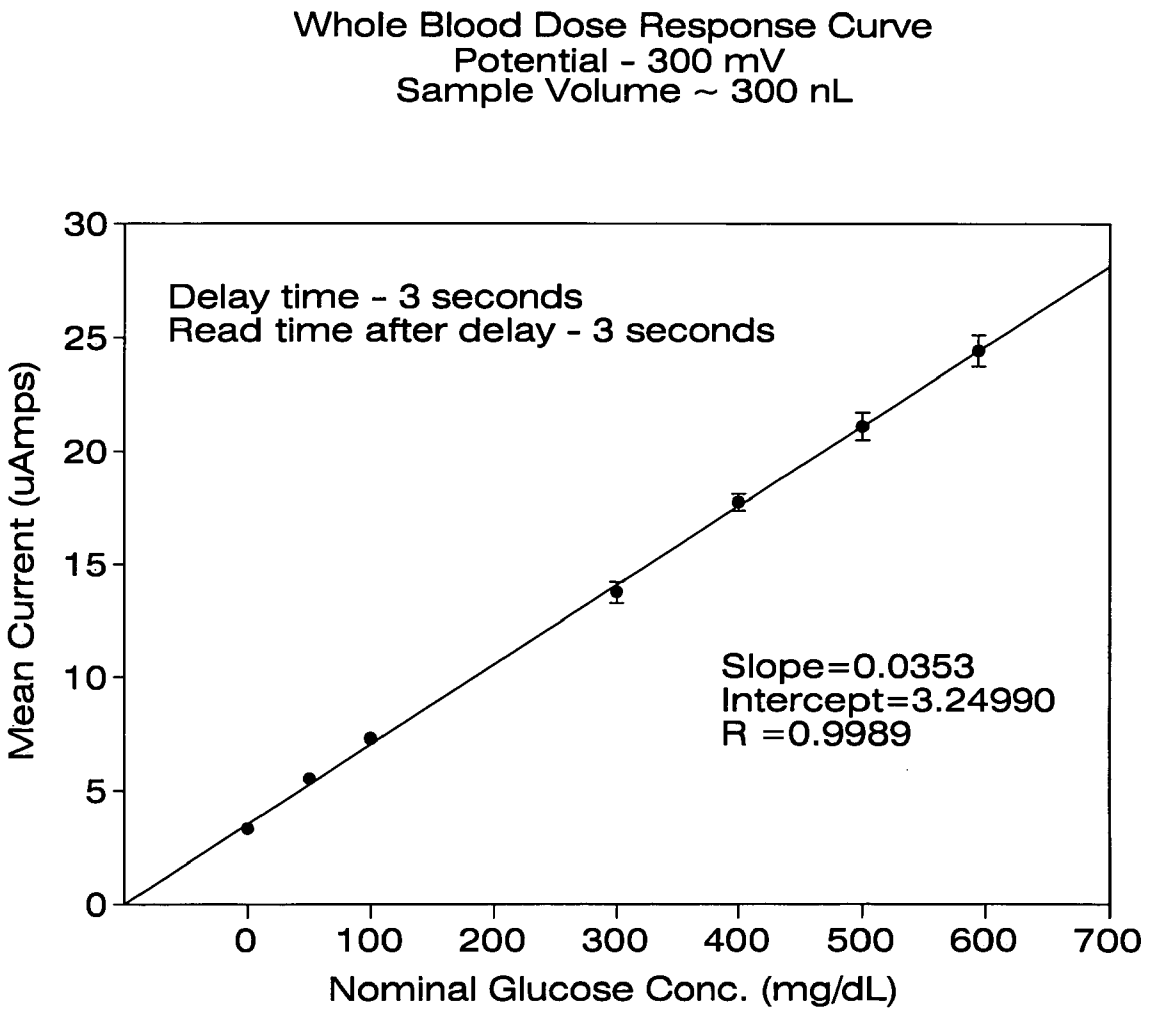
Whole Blood Dose Response Curve
Potential - 300 mV
Sample Volume ~ 300 nL
Delay time - 3 seconds
Read time after delay - 3 seconds
Slope=0.0353
Intercept=3.24990
R =0.9989
Mean Current (uAmps)
Nominal Glucose Conc. (mg/dL)
0
5
10
15
20
25
30
0
100
200
300
400
500
600
700

Fig. 7

METHOD FOR DETERMINING THE CONCENTRATION OF AN ANALYTE IN A LIQUID SAMPLE USING SMALL VOLUME SAMPLES AND FAST TEST TIMES

REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 10/264,785, filed on Oct. 4, 2002, which claims the benefit of U.S. Provisional Patent Application No. 60/332,411, filed on Nov. 16, 2001.

FIELD OF THE INVENTION

The present invention relates to methods for determining the concentration of an analyte in a liquid sample, and particularly to methods using sample volumes of less than 1.5 μl and test times within ten seconds after application of the sample.

BACKGROUND

Electrodes are well known devices which permeate industry, and which, although often very small in size and not particularly visible, can have a significant impact on peoples' lives. Electrodes are used in electronic instruments having many industrial, medical, and analytical applications. To name just a few, they include monitoring and controlling fluid flow, and various types of analytical methods wherein electric current is measured to indicate the presence or concentration of certain chemical species.

With respect to analytical methods, the need for detection and quantitative analysis of certain chemicals found within a larger composition can be important for the chemical and manufacturing industries, as well as biotechnology, environmental protection, and health care industries. Examples of substances that may be analyzed include liquid samples such as tap water, environmental water, and bodily fluids such as blood, plasma, urine, saliva, interstitial fluid, etc.

Many analytical techniques, sometimes referred to as electrochemical detection methods, make use of electrodes as a component of an electrochemical sensor. The sensors are used in combination with electronic apparatuses to precisely detect the presence or concentration of a selected chemical species (analyte) within a substance sample. Techniques that allow the use of miniaturized disposable electroanalytic sample cells for precise micro-aliquote sampling, and self-contained, automatic means for measuring the analysis, can be particularly useful.

Electrochemical detection methods can include amperometric measurement techniques, which generally involve measurement of a current flowing between electrodes that directly or indirectly contact a sample of a material containing an analyte, and studying the properties of the current. The magnitude of the current can be compared to the current produced by the system with known samples of known composition, e.g., a known concentration of analyte, and the quantity of analyte within the sample substance can be deduced. These types of electrochemical detection methods are commonly used because of their relatively high sensitivity and simplicity.

Micro-electrode arrays are structures generally having two electrodes of very small dimensions, typically with each electrode having a common element and electrode elements or micro-electrodes. If "interdigitated" the arrays are arranged in an alternating, finger-like fashion (See, e.g., U.S. Pat. No. 5,670,031). These are a sub-class of micro-electrodes in general. Interdigitated arrays of micro-electrodes, or IDAs, can exhibit desired performance characteristics; for example, due to their small dimensions, IDAs can exhibit excellent signal to noise ratios.

Interdigitated arrays have been disposed on non-flexible substrates such as silicon or glass substrates, using integrated circuit photolithography methods. IDAs have been used on non-flexible substrates because IDAs have been considered to offer superior performance properties when used at very small dimensions, e.g., with feature dimensions in the 1–3 micrometer range. At such small dimensions, the surface structure of a substrate (e.g., the flatness or roughness) becomes significant in the performance of the IDA. Because non-flexible substrates, especially silicon, can be processed to an exceptionally smooth, flat, surface, these have been used with IDAs.

SUMMARY OF THE INVENTION

Whereas micro-electrodes have in the past been used with non-flexible substrates such as silicon, ceramic, glass, aluminum oxide, polyimide, etc., it has now been discovered that micro-electrode arrays, for example, IDAs, can be advantageously useful when disposed on flexible substrates. Moreover, such micro-electrodes, disposed on flexible surfaces, can be prepared using methods that involve flexible circuit photolithography, as opposed to methods relating to integrated circuit photolithography.

An interdigitated array of the invention, disposed on a flexible substrate, can be used generally, in applications where IDAs are known to be usefully employed. In particular embodiments of the invention, the IDAs can be used to construct electrochemical sensors, test cells, or test strips. The sensors can be used with electronic detection systems (sometimes referred to as "test stands") in methods of analyzing sample compositions for analytes. Preferred embodiments of sensors can be disposable, and can include channels or microchannels, preferably a capillary, which facilitates flow of a substance sample into the reaction chamber and in contact with the sensor.

The micro-electrode arrays of the invention can be useful when disposed onto a flexible substrate. In particular, IDAs are shown to be effective at dimensions relatively larger than the dimensions often used for IDAs disposed on non-flexible substrates. Even though they can be relatively larger than IDAs disposed on non-flexible substrates, the inventive IDAs are still able to exhibit performance properties, e.g., signal to noise amplification benefits and steady-state assay profiles, comparable to IDAs having smaller dimensions.

Electrochemical sensors of the invention have been found to provide performance advantages, e.g., relative to commercially available sensors. For sensors used in glucose monitoring, compared to commercially available sensors, the inventive sensors can exhibit improved (shortened) processing periods, e.g., one half second to steady-state after application of the assay potential and 5 seconds to readout, and the ability to get an accurate and precise readout from a relatively small sample of substance, e.g., less than one microliter (μl), preferably a sample volume in the range from about 0.25 to 1.0 μl e.g., from about 0.4 to about 1.0 μl.

The use of larger-dimensioned micro-electrode arrays also allows the significant advantage of fabricating arrays and sensors using relatively less expensive and more efficient flex circuit photolithography processes. These can advantageously incorporate the use of solid materials instead of spin-on liquid materials, e.g., one or more of a solid

photoresist or a solid coverlay, instead of liquid materials typically used in integrated circuit photolithography.

An aspect of the invention relates to micro-electrodes used in combination with a flexible substrate. The array can include a working electrode and a counter electrode, each including a common lead and commonly-connected electrode elements, for example with the electrode elements being arranged in a substantially-parallel, alternating fashion. Preferred dimensions for micro-electrodes can be, e.g., feature size or width of electrodes (W_e) in the range from 15 or 20 or 25 μm, up to about 100 μm, more preferably from greater than or about 25 or 30 μm to about 50 μm. Preferred spacing between electrodes (W_g) can also be in the range from about 15 to about 50 μm, more preferably from greater than or about 20 or 25 μm to about 45 μm.

Another aspect of the invention relates to an electrochemical sensor comprising an array of micro-electrodes disposed on a flexible substrate. The sensor can further include a chemical coating disposed on the array to facilitate practice of electrochemical detection methods.

Yet another aspect of the invention relates to a method of detecting an analyte using an array of micro-electrodes of the invention, e.g., using an electrochemical sensor comprising an interdigitated array disposed proximal to a flexible substrate. Such a method can include certain of the following steps. A sensor is provided which comprises micro-electrodes proximal to a flexible substrate, and a chemical coating proximal to the micro-electrodes; the coating comprises a compound reactive to produce an electroactive reaction product. The coating is contacted with a substance comprising an analyte, allowing the analyte to react with chemical components of the coating to produce an electroactive reaction product. Electric properties of the coating can be measured, and the electric properties can be correlated to the amount of electroactive reaction product, and to the amount of analyte.

Still another aspect of the invention relates to a method of preparing a micro-electrode, including the step of disposing the micro-electrode onto a flexible substrate.

More particularly, the present invention comprises a method for determining the concentration of glucose in a blood sample. The method utilizes a disposable test strip having a capillary-fill chamber including a working electrode and a counter and/or reference electrode and a reagent. The reagent includes an enzyme and a mediator, and reacts with glucose to produce an electroactive reaction product. The method involves providing a blood sample to the capillary chamber and detecting the presence of the blood sample in the capillary chamber. Within 10 seconds of detecting the presence of the blood sample, the glucose concentration is determined.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows an embodiment of an interdigitated array of the invention.

FIGS. **2** and **2**A each show a top view of a sensor of the invention.

FIG. **3** shows a top view of a sensor of the invention.

FIG. **4** shows a side view of a sensor of the invention.

FIG. **5** shows a side view of a sensor of the invention.

FIG. **6** shows a perspective view of a dissembled sensor of the invention.

FIG. **7** shows data of assay current versus blood glucose level.

DETAILED DESCRIPTION

An embodiment of the present invention is directed to arrays of micro-electrodes, e.g., an interdigitated array of electrodes (sometimes referred to as "microband" electrodes) used in combination with a flexible substrate.

An array of micro-electrodes includes two electrodes, referred to as the working electrode and the counter electrode, electrically insulated from one another.

Micro-electrodes, as distinguished from other electrodes generally, are understood in the electronic and biosensor arts. In analyzing a liquid sample using electrodes and electronic equipment and techniques, the size and spacing of electrodes can affect whether diffusion of an analyte through the sample to an electrode occurs by a planar or non-planar path. Micro-electrode arrays are of a size and spacing such that in detecting chemical species of a solution, the species will diffuse toward or approach an electrode of the micro-electrode array in a non-planar fashion, e.g., in a curved or hemispherical path of diffusion. In contrast, non-microelectrodes, i.e., "macro-electrodes," cause diffusion of an analyte through a solute according to a substantially planar path. It is also understood that some electrode configurations can cause diffusion to take place by a mix of planar and non-planar paths, in which case the electrodes can be considered a micro-electrode array, especially if the diffusion occurs predominantly (e.g., greater than 50%) according to a non-planar path, or if the size of the electrodes is less than 100 μm, e.g., less than 50 μm.

The electrodes of a micro-electrode array are positioned near each other in an arrangement that will result in non-planar diffusion as described. The arrangement of the electrodes can be any arrangement that results in such diffusion, with a working and a counter electrode being substantially evenly spaced from each other. One electrode may be arranged into a shape or figure or outline that will produce interstices within which the second electrode may be placed. For instance, one electrode can be arranged as an increasing radius, substantially circular spiral, with a continuous, long and narrow interstitial area being created between each successively larger revolution of electrode. The other electrode can be positioned in the interstitial area between revolutions, while the electrodes remain insulated from one another. The width and spacing of the electrodes can be arranged to result in micro-electrode array performance.

According to other forms of such micro-electrode arrays, the spiral may not be substantially circular, but could include linear, square, angled, or oblong or oval features. Or, the electrodes could be arranged in any other geometric form whereby the electrodes are placed adjacent to each other and within the other's respective interstitial area, e.g., by following a similar path separated by a substantially uniform gap.

In one particular embodiment, the micro-electrode can be arranged into an interdigitated array, meaning that at least a portion of electrode elements of the working electrode are placed substantially parallel to and in alternating succession with at least a portion of the electrode elements of the counter electrode, e.g., in an alternating, "finger-like" pattern. Such interdigitated micro-electrode arrays include electrode elements (sometimes referred to as "fingers") and a common element ("contact strip") which commonly connects the electrode elements.

The components of the electrodes may be made of any conductive material, including those known and conventionally used as electrode materials, particularly including materials known in the flexible circuit and photolithography arts.

These can include, for example, carbon, noble metals such as: gold, platinum, palladium, alloys of these metals, potential-forming (conductive) metal oxides and metal salts, as well as others.

The electrodes and their components can be of dimensions, meaning the width of the electrode components as well as the separation between components, that can provide an array with useful properties, e.g., useful or advantageous capabilities with respect to contacting a substance or measuring electrical properties. Advantageously, interdigitated arrays can be prepared at dimensions that allow for contact with and measurement of electrical properties of a relatively small sample of a substance.

In preferred embodiments of the invention, each electrode element can independently have a width (W_e) in the range from greater than 15 micrometers (μm) to about 50 μm, with the range from greater than or about 20 or 25 μm to about 40 μm being particularly preferred. The separation between electrode components (W_g), especially the separation between alternating electrode elements, can also preferably be in the range between about 15 micrometers and about 50 μm, with the range from greater than or about 20 or 25 μm to about 40 μm being particularly preferred. The total area of an electrode (meaning the area of the fingers but not the common element) can be chosen depending on these dimensions, on the use intended for the electrode, on the desired current level intended to pass through the electrode, and on the desired number of electrode elements. An exemplary area of an electrode having 10 electrode elements can be in the range from about 0.1 to about 0.5 square millimeters, (for example 10 electrode fingers having dimensions of 50 μm by 1 mm), e.g., from about 0.2 to 0.3.

The thickness of the electrode components can be sufficient to support a desired electric current. Exemplary thicknesses can be in the range from about 30 to 200 nanometers (nm), with a preferred thickness being about 100 nm.

The electrodes can independently have a number of interdigitated electrode elements sufficient to provide utility, e.g., allowing contact with a substance to measure its electrical behavior. Conventionally, the array can have substantially the same number (equal, plus or minus one) of electrode elements in the working electrode as are in the counter electrode, allowing the electrode elements to be paired next to each other in an alternating sequence. In some preferred embodiments of the array, such as in some of the applications described below for electrochemical sensors, each electrode of an array may typically have from about 4 to about 30 electrode elements.

FIG. **1** illustrates an embodiment of an array of the invention. Working electrode **2** and counter electrode **4** are arranged as an interdigitated array on flexible substrate **10**. (The figure is not to scale and its dimensions, as well as the dimensions of the other figures, should not be construed to limit the invention). The working and counter electrodes include common strips **6***a* and **6***b*, respectively, which can be connected to electrically conductive means (e.g., "connectors," "pads," or "leads," etc.) for connecting the electrodes to an external circuit. In the illustrated example, the working electrode includes electrode elements **8***a* connected to common strip **6***a*, and the counter electrode includes electrode elements **8***b* connected to common strip **6***b*.

According to the invention, the interdigitated array is disposed proximal to, e.g., on, a flexible substrate. To act as a flexible substrate, a material must be flexible and also insulating, and is typically relatively thin. The substrate should be capable of adhering components of an IDA, or additional components of a sensor, to its surface. Such thin, insulative, flexible substrates are known in the art of flexible circuits and flex circuit photolithography. "Flexible substrates" according to the present disclosure can be contrasted to non-flexible substrates used in integrated circuit (IC) photolithography but not in flexible circuit photolithography. Examples of non-flexible substrates used in IC photolithography include silicon, aluminum oxide, and other ceramics. These non-flexible substrates are chosen to be processable to a very flat surface. Typical flexible substrates for use in the invention are constructed of thin plastic materials, e.g., polyester, especially high temperature polyester materials; polyethylene naphthalate (PEN); and polyimide, or mixtures of two or more of these. Polyimides are available commercially, for example under the trade name Kapton®, from I.E. duPont de Nemours and Company of Wilmington, Del. (duPont). Polyethylene naphthalate is commercially available as Kaladex®, also from duPont. A particularly preferred flexible substrate is 7 mil thick Kaladex® film.

Interdigitated arrays of the invention can be used in applications generally known to incorporate electrodes, especially applications known to involve interdigitated arrays of electrodes. Various applications are known in the arts of electronics and electrochemistry, including applications relating to process and flow monitoring or control, and chemical analytical methods. The arrays may be particularly useful as a component of an electrochemical sensor, where there is added value, benefit, or cost efficiency, to the use of a flexible substrate, or where there is value, benefit, or cost efficiency in having an interdigitated array of dimensions relatively larger than the dimensions of interdigitated arrays conventionally disposed on non-flexible substrates.

An interdigitated array of the invention can, for example, be included in an electrochemical sensor (sometimes referred to as a "biosensor" or simply "sensor") used in electrochemical detection methods. Electrochemical detection methods operate on principles of electricity and chemistry, or electrochemistry, e.g., on principles of relating the magnitude of a current flowing through a substance, the resistance of a substance, or a voltage across the substance given a known current, to the presence of a chemical species within the substance. Some of these methods can be referred to as potentiometric, chronoamperometric, or impedence, depending on how they are practiced, e.g., whether potential difference or electric current is controlled or measured. The methods and sensors, including sensors of the invention, can measure current flowing through a substance due directly or indirectly to the presence of a particular chemical compound (e.g., an analyte or an electroactive compound), such as a compound within blood, serum, interstitial fluid, or another bodily fluid, e.g., to identify levels of glucose, blood urea, nitrogen, cholesterol, lactate, and the like. Adaptations of some electrochemical methods and electrochemical sensors, and features of their construction, electronics, and electrochemical operations, are described, for example, in U.S. Pat. Nos. 5,698,083, 5,670,031, 5,128,015, and 4,999,582, each of which is incorporated herein by reference.

Oftentimes, a compound of interest (analyte) in a substance is not detected directly but indirectly, by first reacting the analyte with another chemical or set of chemicals proximal to or in contact with an IDA. The reaction produces an electroactive reaction product that is electrochemically detectable and quantifiable by applying a potential difference between the counter and working electrodes and measuring the magnitude of the current produced. This allows measurement of the amount of electroactive reaction product

generated by the first reaction, and correlation of that measurement to the amount of analyte in the sample substance.

An example of such a method involves the catalytic use of an enzyme, and is sometimes referred to as enzymatic amperometry. These methods can use an interdigitated array of electrodes coated with a chemical coating that contains a chemical compound reactive to produce an electroactive reaction product. (The chemical compound reactive to produce an electroactive reaction product is sometimes referred to herein as a "mediator.") Upon contacting the coating with a sample that contains an analyte, analyte reacts with chemical compounds of the coating to generate electroactive reaction product. This electroactive reaction product can be electronically detected, measured, or quantified, by applying a potential difference between the electrodes and measuring the current generated by the electrooxidation of the mediator at the working electrode. By calibrating the system's behavior using known substances and concentrations, the electrical behavior of the system in the presence of a sample substance of unknown composition can be determined by comparison to the calibration data.

The sensor of the invention may be used in amperometric applications, e.g., enzymatic amperometric applications, if disposed on the array is a coating of useful chemistry, including e.g., an enzyme and a mediator. When a sample containing an analyte is contracted with the coating, the analyte, enzyme, and the mediator participate in a reaction, wherein the mediator is either reduced (receives at least one electron) or is oxidized (donates at least one electron). Usually, in this reaction, the analyte is oxidized and the mediator is reduced. After this reaction is complete, an electrical potential difference can be applied between the electrodes. The amount of reducible species and the applied potential difference must be sufficient to cause diffusion-limited electrooxidation of the reduced form of the mediator at the surface of the working electrode. The IDA electrode configuration of the sensor places the working electrode fingers in close proximity to counter electrode fingers. Mediator electrooxidized at the working electrode can therefore diffuse rapidly to the adjacent counter electrode via radial diffusion where it is once again reduced. Likewise, oxidized mediator reduced at the counter electrode can migrate to the working electrode for electrooxidation to the oxidized form. This migration between the fingers produces a constant or "steady state" current between the electrodes. After a short time delay, this steady state current is measured and correlated to the amount of analyte in the sample.

The chemistries of the first and second reactions can be of any nature effective to produce the electroactive reaction product of the first reaction, to detect or quantify the electroactive reaction product during the second reaction, and to allow correlation of the amount of electroactive reaction product with the presence or concentration of analyte in the original sample.

In general, a typical first reaction can be an oxidation/reduction sequence, preferably occurring without the need for a chemical potential across the electrodes. It can be desirable for this reaction to favor maximum, preferably complete conversion of the analyte, and to proceed as quickly as possible. Often this reaction is catalyzed, e.g., enzymatically. Such reaction schemes and their application to enzymatic amperometry are known. See, e.g., U.S. Pat. No. 5,128,015; European Patent Specification EP 0 406 304 B1; and Aoki, Koichi, *Quantitative Analysis of Reversible Diffusion-Controlled Currents of Redox Soluble Species at Interdigitated Array Electrodes Under Steady-State Conditions*, J. Electroanal. Chem. 256 (1988) 269–282. An example of a useful reaction scheme can be the reaction of a component of a bodily fluid, e.g., glucose, with an enzyme and a cofactor, in the presence of a mediator, e.g., an oxidizer, to produce an electroactive reaction product.

The chemistry of a first reaction scheme of any chosen electrochemical detection method can be chosen in light of various chemical factors relating to the system, including the identity of the analyte and of the sample substance. Even then, for a given analyte or substance, various different reactive components may be useful in terms of a catalyst (often, a variety of enzymes will be useful), co-reactants (e.g., a variety of mediators may be useful), and cofactors (if needed, a variety may be useful). Many such reaction schemes and their reactive components and reaction products are known, and examples of a few different enzymes include those listed in Table 1.

Analyte	Enzymes	Redox Mediator (Oxidized Form)	Additional Mediator
Glucose	Glucose dehydrogenase and Diaphorase	Ferricyanide, osmium (III)-(bipyridyl)-2-imidazolyl-chloride, Meldola blue, $[Ru(NH_3)_5MeIm]Cl_3$ $[OS(III)(NH_3)_5pyz]_2(SO_4)_3$, NITROSO analine derivatives	
Glucose	Glucose oxidase	(see above)	
Cholesterol	Cholesterol esterase and Cholesterol oxidase	(see glucose)	2,6-Dimethyl-1, 4-Benzoquinone, 2,5-Dichloro-1, 4-benzoquinone, or phenazine ethosulfate
HDL Cholesterol	Cholesterol esterease and Cholesterol oxidase	(see glucose)	2,6-Dimethyl-1, 4-Benzoquinone, 2,5-Dichloro-1, 4-benzoquinone, or phenazine ethosulfate
Triglycerides	Lipoprotein lipase, Glycerol kinase, Glycerol-3-phosphate oxidase	(see glucose)	Phenazine methosultate, phenazine ethosulfate.
Triglycerides	Lipoprotein lipase, Glycerol kinase, Glycerol-3-phosphate dehydrogenase and Diaphorase	(see glucose)	Phenazine methosultate, phenazine ethosulfate.
Lactate	Lactate oxidase	(see glucose)	2,5-Dichloro-1, 4-benzoquinone
Lactate	Lactate dehydrogenase and Diaphorase	(see glucose)	
Lactate Dehydrogenase	Diaphorase	(see glucose)	
Pyruvate	Pyruvate oxidase	(see glucose)	
Alcohol	Alcohol oxidase	(see glucose)	
Alcohol	Alcohol dehydrogenase and Diaphorase	(see glucose)	

-continued

Analyte	Enzymes	Redox Mediator (Oxidized Form)	Additional Mediator
Uric acid	Uricase	(see glucose)	
3-Hydroxy-butric acid (ketone bodies)	3-Hydroxybutyrate dehydrogenase and Diaphorase	(see glucose)	

A mediator can be any chemical species (generally electroactive), which can participate in a reaction scheme involving an enzyme, an analyte, and optionally a cofactor (and reaction products thereof), to produce a detectable electroactive reaction product. Typically, participation of the mediator in this reaction involves a change in its oxidation state (e.g., a reduction), upon interaction with any one of the analyte, the enzyme, or a cofactor, or a species that is a reaction product of one of these (e.g., a cofactor reacted to a different oxidation state). A variety of mediators exhibit suitable electrochemical behavior. A mediator can preferably also be stable in its oxidized form; may optionally exhibit reversible redox electrochemistry; can preferably exhibit good solubility in aqueous solutions; and preferably reacts rapidly to produce an electroactive reaction product. Examples of suitable mediators include benzoquinone, medula blue, other transition metal complexes, potassium ferricyanide, and nitrosoanalines, see U.S. Pat. No. 5,286,362. See also Table 1.

To describe an example of an oxidation/reduction reaction scheme that is known to be useful for detecting glucose in human blood, a sample containing glucose can react with an enzyme (e.g., Glucose-Dye-Oxidoreductase (Gluc-Dor)) and optionally a cofactor, (e.g., pyrrolo-quinoline-quinone), in the presence a redox mediator (e.g., benzoquinone, ferricyanide, or nitrosoanaline derivatives), to produce the oxidized form of the analyte, gluconolactone, and the reduced form of the redox mediator. See U.S. Pat. No. 5,128,015. Other examples of reaction schemes are known, and are typically used in methods designed to detect a specific analyte, e.g., cholesterol, urea, etc.

After the reaction is complete, a power source (e.g., battery) applies a potential difference between the electrodes. When the potential difference is applied, the amount of oxidized form of the redox mediator at the counter electrode and the potential difference must be sufficient to cause diffusion-limited electrooxidation of the reduced form of the redox mediator at the working electrode surface. In this embodiment, the close proximity of the counter and working electrode fingers in the IDA electrode configuration aids in the fast radial diffusion of the reduced and oxidized redox mediator between the electrodes. Recycling of the mediator between the electrodes and their subsequent oxidation and reduction on the electrodes generates a constant or "steady state" assay current. This steady state assay current is measured by a current measuring meter.

The measured current may be accurately correlated to the concentration of analyte in the sample when the following requirements are satisfied:

1) the rate of oxidation of the reduced form of the redox mediator is governed by the rate of diffusion of the reduced form of the redox mediator to the surface of the working electrode; and

2) the current produced is limited by the oxidation of the reduced form of the redox mediator at the surface of the working electrode.

In the preferred embodiment, these requirements are satisfied by employing a readily reversible mediator and by using a mixture of amounts of mediator and other components of the chemical layer to ensure that the current produced during diffusion limited electrooxidation is limited by the oxidation of the reduced form of the mediator at the working electrode surface. For current produced during electrooxidation to be limited by the oxidation of the reduced form of the mediator at the working electrode surface, the amount of reducible species at the surface of the counter electrode must always exceed the amount of the reduced form of the redox mediator at the surface of the working electrode.

An example of a reaction scheme relates to the detection of glucose using ferricyanide and Glucose-Dye-Oxidoreductase (Glur-Dor). The electroactive reaction product of the enzymatic reaction between glucose and the enzyme is the reduced mediator, ferrocyanide. The ferrocyanide is electrooxidized at the working electrode back to ferricyanide. One mole of oxidized redox mediator is reduced at the counter electrode for every mole of reduced redox mediator oxidized at the working electrode. Ferricyanide electrooxidized at the working electrode, diffuses to the counter electrode, and the ferrocyanide produced at the counter electrode can rapidly diffuse to the working electrode where it is again oxidized. A "quasi-steady state" concentration gradient is established between the counter and working electrode pairs resulting in generation of a constant quasi-steady state current at the working electrode.

The magnitude of the current, preferably as measured at a quasi-steady-state condition, can be correlated to the amount of electroactive reaction product present in the coating, and consequently, to the amount of analyte in the sample.

The chemical coating should allow diffusion of analyte into the coating, followed by reactions as described. The coating can include materials which can contain the reactive chemical components, which allow reaction between the components to product an electroactive reaction product, which allow necessary diffusion of chemical components, and which can support a current passing through the coating based on the concentration of electroactive reaction product. Typically, the coating can be made up of a binder that contains a set of chemicals which react to produce an electroactive reaction product. The chemicals generally include a mediator and necessary enzymes and cofactors. Such a coating can also contain a variety of additional components to make the coating operative and suitable for processing, including specific components listed above as well as surfactants, film formers, adhesive agents, thickeners, detergents, and other ingredients and additives that will be understood by an artisan skilled in the electrochemical sensor art.

The binder can provide integrity of the coating while allowing diffusion of the different components of the reaction scheme, reaction between the reactive components, and movement of reactive components and products sufficient to produce a quasi-steady-state concentration gradient of mediator and electroactive reaction product and thereby establish a stable or quasi-steady-state current between the electrode pairs. Exemplary binders can include gelatin, carrageenan, methylcellulose, polyvinyl alcohol, polyvinylpyrrolidone, alignate, polyethylene oxide, etc.

A sensor according to the invention can be understood to include a micro-electrode disposed on a flexible substrate, optionally including a chemical coating, and further including any immediate appurtenance necessary to use the sensor

in an electronic system or apparatus (e.g., test stand) designed, for example, for use in an electrochemical detection method. A sensor can include the interdigitated array disposed on a flexible substrate, with additional components to independently connect each of the separate electrodes to a different voltage, e.g., electrical connectors, leads, or pads. In some circumstances, the sensor may include a reference electrode provided on the same or a different substrate and electrically insulated from the interdigitated array. The sensor may also include components to direct flow of a sample substance into contact with the IDA, e.g., a vessel, channel, microchannel, or capillary. A particularly preferred embodiment of the sensor includes a microchannel or capillary, most preferably a capillary, which directs flow of a sample substance into the reaction chamber and over the IDA (e.g., a coated IDA).

A capillary can be included in a sensor to facilitate analysis of a small volume of a sample substance by precisely directing the flow of a volume of sample over the IDA, preferably in a short period of time. Analysis of relatively small volumes of a sample substance can be accomplished, at least in part, due to the signal amplification features of the IDA.

Preferred dimensions of a capillary for what can be referred to as a "low volume sensor configuration," can be in the range of 0.025 mm to 0.2 mm (depth), preferably about 0.125 mm (depth), ×1 mm (width)×3 mm (length), resulting in a capillary chamber requiring a relatively small volume of sample, e.g., less than 400 nanoliters (nL). The volume of the chamber can preferably be such that a low volume sample of a substance can be directed into or through the chamber for analysis. Chamber volumes will vary depending on the type of analyte being studied, and even its concentration of an analyte. (Blood samples of different hematocrits will dispense differently into a capillary.) Exemplary chamber volumes can be in the range from about 100 to 300 nanoliters for glucose analysis in interstitial fluid, and from about 250 to 400 nanoliters for glucose analysis applications in the whole blood. In the most preferred embodiments of the sensor, including a capillary, the capillary may have a vent to facilitate flow of a sample substance into the capillary chamber by equalizing pressure between the interior and exterior of the chamber.

The sensor of the invention can include these and other features, and, especially if an embodiment is disposable, can be referred to as a "test strip" or a "test cell." The term "disposable" refers to sensors designed or sold for a single use, after which they are to be discarded or otherwise stored for later disposal.

Capillaries may be fabricated as a component of a sensor, using photolithographic methods, e.g., as described infra.

An example of a sensor construction is shown in FIG. **2**, according to the preferred embodiment. The figure shows sensor **20**, including an interdigitated array of electrodes **22** disposed on flexible substrate **24**. The electrodes are connected to electrically-conductive connectors **26** which include portions **28** that can be identified as pads, located on the surface of the flexible substrate, where they are available to be contacted to an external electronic circuit such as a testing apparatus. The connectors also include connector portions **30**, which connect electrode elements at the array to the pads, and which may typically be covered by an insulating layer. FIG. **2***a* shows a close-up of array **22**, showing that electrodes attached to each of connectors **26** are arranged in an inter digitated fashion (as shown in FIG. **1**).

FIG. **3** shows different details of a sensor of the invention. FIG. **3** shows sensor **20** comprising flexible substrate **24**, an array of interdigitated electrodes **22**, and connectors and pads. Non-conductive layer **32** is disposed over the substrate and connector portions **30** of the connectors **26**, over portions of the array **22**, and not over a rectangular capillary portion including some of the substrate and an intersection of array **22**; this rectangular portion defines capillary chamber **34**. (A chemical coating, not shown in this figure, is preferably disposed over the array, within the capillary chamber.) Foil **36** covers a rectangular portion of the sensor, including portions of the non-conductive layer **32**, and a portion of capillary chamber **34**, except for air vent **38**. This embodiment is shown from one side in FIG. **4**, and from another side in FIG. **5**. FIG. **5** specifically illustrates substrate **24**, array **22**, non-conductive layer **32**, which defines chamber **34**, and foil **36**. FIG. **5** additionally includes coating **40** disposed on array **22**, within the capillary.

FIG. **6** illustrates an exploded view of a sensor of the invention. The sensor **20** includes flexible substrate **24**; a conductive film **40** patterned with an interdigitated array of electrodes **22** and connectors **26** which include pad portions **28** and connecting portions **30**, an insulating material **32** which defines the depth and dimensions of capillary chamber **34**, a chemical coating **40** disposed in the capillary chamber **34**, and top foil **36** coated with a hydrophilic adhesive layer **42**.

The array of the invention, in various embodiments such as a sensor, can be used in electrochemical detection methods, including those using the principles and specific methods described above, and others. Such methods employ the array disposed on a flexible substrate, preferably further including a chemical coating contacting the array.

Upon contacting the coating with a sample containing analyte, analyte generally diffuses into the coating at a rate dependant on factors such as the chemical composition of the coating and the chemical identity of the analyte. Generally, the chemical coating will be at least partly solubilized or hydrated by the sample substance. For a method to provide the quickest read time (the time following contact with a substance sample, when a reading of the concentration of analyte in the substance is available), it is desirable that the analyte diffuse quickly into the coating, and thereafter quickly and completely react to produce an electroactive reaction product. The period during which this occurs can be reduced by operating on a relatively small volume of sample, and by using a sensor having a relatively small amount of chemical coating to be solubilized or hydrated.

The time from when the substance containing the analyte is contacted with the chemical coating until an assay potential is applied to the array, and during which the analyte diffuses into the coating and reacts to produce an electroactive reaction product, can be referred to as the "delay period". This period can be any amount of time necessary for the above occurrences to transpire, is preferably minimized, and in some embodiments can be less than about 10 seconds, preferably in the range from about 2 to 6 seconds.

After a delay period, the electric properties of the coating can be measured. By chronoamperometric methods, or by potentiometric methods, as will be appreciated by the skilled artisan, either the current or the applied potential can be controlled, and any of the related current, resistance, or voltage can be measured and correlated to amounts of electroactive reaction product and analyte. The magnitude of the current, or alternatively potential difference or the resistance of the chemical coating, can be measured using an external circuit connected to the sensor electrodes.

As an example, according to chronoamperometric methods, a potential ("assay potential") can be applied across the

electrodes, inducing a current ("assay current") to flow through the coating. The potential should be enough to cause reduction or oxidation of the redox products formed in the first step of a binary reaction scheme (e.g., as described above), but should not be sufficient to cause other electrochemical reactions or to otherwise cause significant current to flow through the coating. The assay potential can be chosen depending on the redox mediator chosen, factors relating to the electrochemical detection method, the electrochemical system and reaction scheme, and the general capabilities of the sensor. A typical potential can be in the range of a few to several hundred millivolts, e.g., from about 100 to 500, preferably 200 to 400 millivolts.

A measured current can initially exhibit a spike to a relatively elevated level, and can then descend to a steady-state current based on a quasi-steady-state concentration gradients and a recycle reaction loop of the mediator and electroactive reaction product. Preferably, the magnitude of the current can be measured at a time when current flowing through this system has approached a plateau, based on quasi-steady-state concentration gradients. The period of time starting with application of the assay potential and lasting to the plateau or near-steady-state current can be referred to as the "assay period." Steady-state assay currents can occur within various such time periods, depending upon the reaction scheme, the chemistries of its components, etc. In the practice of the invention, assay periods of less than one minute are preferred, e.g., less than 30 seconds, and assay periods of even shorter duration, less than 10 seconds, are most preferred. The assay profile (the profile of the assay current over time) can be to some extent controlled by changing the spacing between electrode elements in the array; increased spacing between electrode elements can result in a longer time interval between assay potential application and formation of the steady state assay currents.

Assay currents exhibited by exemplary sensors of the invention can be any current that will function in an electrochemical detection method. For the sensors of the invention, any useful current can be used, preferably with a range between a lower end in the nanoamp range (e.g., between 20 to 25 nanoamps) up to the microamp range e.g., 100 microamps, being an exemplary working range, e.g., at the steady state current plateau. Typical steady state assay currents can be in the range from below one microamp up to around 100 microamp, preferably from about 0.5 to about 25 microamps. In an embodiment of the invention useful for detecting glucose content of a blood sample, the current response (steady state assay current) in this range has been found to be linear with respect to the concentration of glucose in the sample, particularly for glucose concentrations in the range from about 0 to 600 milligrams per deciliter (mg/dL).

Sensors of the invention may be used in cooperation with electronic or computerized systems and apparatuses, and in combination with methods for identifying analytes and measuring concentration of analytes within a substance sample. For example, a sensor can be used with a VXI or Biopotentiostat test stand built from components purchased from National Instrument Corp., Austin, Tex. In this context, the method of the invention can be practiced with a delay period of around 3 seconds, an assay potential of about 300 millivolts, and an assay period which, although variable, can preferably be in the range from about 1.5 to 2 seconds after applying the assay potential.

The sensors can be used in such a method to detect and quantify the concentration of an analyte within a sample substance. The analyte can be chosen from various chemical compounds present within any of a large variety of substances, generally fluids. Examples of analytes include glucose, cholesterol, urea, and the like. Examples of substances containing the analyte include bodily fluids such as blood, urine, and interstitial fluid; water such as environmental water, ground water, waste water, etc.

In some embodiments of the invention, analytes can be detected at very low concentrations, for example glucose can be measured at concentrations as low as 0.5 mg/dL (5 ppm) in blood using ferricyanide as the mediator.

The use of an array or sensor of the invention offers certain practical advantages. For instance, a flexible substrate can be used in combination with relatively larger-dimensioned electrodes, including electrode components of increased size (e.g., width) as well as increased spacing between them. Lower sample volumes can independently decrease the time of the delay period. A shorter delay period in combination with an expedited formation of a quasi-steady-state region of the assay current produces a quicker read time. In the practice of the invention, read times of less than 10 seconds have been achieved, with a read times in the range from about 4 and 5 seconds being preferred.

Test cells and test strips according to the invention allow for controlled volumes of blood to be analyzed without pre-measuring. Insertion of the test cell into an electronic or computer-controlled apparatus (referred to generally as a test stand) permits automatic functioning and timing of the reaction and analysis of the sample. This allows for patient self-testing with a very high degree of precision and accuracy. The method, the sensor or test cell, and the apparatus, are designed to provide self-monitoring by a patient of important bodily fluids, e.g., blood glucose levels. The sensor is used to control the sample volume and reaction media, to provide precise, accurate, and reproducible analysis. Preferably, disposable test strips or test cells can be used in combination with a portable electrochemical testing meter.

The preferred embodiment of the present invention uses a micro-electrode array consisting of interdigitated microband electrodes as described above. Although this arrangement leads to the aforementioned re-cycling of redox products between narrowly separated working and counter electrodes, this is not a strict requirement for successful practice of the invention. An alternative embodiment is the provision of an array of more general micro-electrodes to act as the working electrode structure. These may be microbands that are not interdigitated with the counter electrode, or micro-disks, also not closely spaced with the counter electrode. In this case the width or diameter of the working electrode bands or disks should be of such a dimension as to allow for some degree of radial or spherical diffusion to the working electrode surfaces. Typically, this dimension should be in the range of 5 to 50 μm, and most preferably 10 to 50 μm for the case of aqueous systems such as encountered with a sensor used for the assay of biological fluids. In both cases the counter electrode is provided at a distance from the working array that is generally larger than the smallest dimension of the working electrodes.

In these embodiments, specific recycling of redox species between the working and counter electrodes is not observed in the same way as in other described embodiments, and assay current magnitudes are consequently reduced. Nevertheless, the effect of radial or spherical diffusion to working micro-electrode structures can still be observed as current densities that are greater than that predicted from linear diffusion alone. Although reduced in magnitude, and not approaching quasi-steady-state as displayed by the preferred

embodiments, it is still possible to measure dose responses to the analyte in question (e.g. glucose) when the same reagent as described above is disposed on the micro-electrode array.

Micro-electrode arrays of the invention can be disposed onto a flexible substrate using various methods useful for disposing electronic components onto substrates, especially flexible substrates. A variety of such methods are generally known for fabrication of different types of circuitry, and include specific techniques of dry-coating, lamination, spin-coating, etching, and laser ablation. One or more of the following generalized methods may be specifically useful to prepare microelectrode arrays according to the invention.

One method of preparing a micro-electrode array as described herein, e.g., an IDA, is by the use of laser ablation techniques. Examples of the use of these techniques in preparing electrodes for biosensors are described in U.S. patent application Ser. No. 09/866,030, "Biosensors with Laser Ablation Electrodes with a Continuous Coverlay Channel" filed May 25, 2001, and in U.S. patent application Ser. No. 09/411,940, entitled "Laser Defined Features for Patterned Laminates and Electrode," filed Oct. 4, 1999, both disclosures incorporated herein by reference.

In general, laser ablative techniques use a laser to cut or mold a material. According to the invention, micro-electrodes can be prepared using ablative techniques, e.g., by ablating a multi-layer composition that includes an insulating material and a conductive material, e.g., a metallic laminate of a metal layer coated on or laminated to an insulating material. The metallic layer may contain pure metals or alloys, or other materials which are metallic conductors. Examples include aluminum, carbon (such as graphite), cobalt, copper, gallium, gold, indium, iridium, iron, lead, magnesium, mercury (as an amalgam), nickel, niobium, osmium, palladium, platinum, rhenium, rhodium, selenium, silicon (such as highly doped polycrystalline silicon), silver, tantalum, tin, titanium, tungsten, uranium, vanadium, zinc, zirconium, mixtures thereof, and alloys or metallic compounds of these elements. Preferably, the metallic layer includes gold, platinum, palladium, iridium, or alloys of these metals, since such noble metals and their alloys are unreactive in biological systems. The metallic layer may be any thickness but preferably is 10 nm to 80 nm, more preferably 20 nm to 50 nm.

In the laser ablation process, the metallic layer may be ablated into a pattern of micro-electrodes. The patterned layer may additionally be coated or plated with additional metal layers. For example, the metallic layer may be copper, which is then ablated with a laser, into an electrode pattern. The copper may be plated with a titanium/tungsten layer, and then a gold layer, to form desired micro-electrodes. Preferably, however, in some embodiments, only a single layer of gold is used. One example of a useful metallic laminate is a polyester or other flexible substrate such as a Kaladex film, coated with a layer of gold, preferably about 5 mils in thickness.

The conductive material is ablated with the laser to leave a micro-electrode array. Any laser system capable of ablation of the conductive material will be useful. Such laser systems are well known and commercially available. Examples include excimer lasers, with a pattern of ablation controlled by lenses, mirrors, or masks. A specific example of such a system is the LPX-400, LPX-300, or LPX-200, both from LPKF LASER ELECTRONIC, GMBH of Garbsen, Germany.

One specific example of a process for laser ablation is as follows. Sheets of sensor traces are fabricated in a MicroLineLaser 200-4 laser system (from LPKF). The system chamber includes a vacuum platen atop of a LPKF-HS precision positioning X,Y table, laser mirrors and optics, and a quartz/chromium photomask (International Phototool Company, Colorado Springs, Colo.) with the sensor components subdivided into rectangular fields on the mask. Photomask positioning, X,Y table movement and laser energy are computer controlled. Sheets of metal laminate 22 cm×22 cm in dimension are placed into the chamber onto the vacuum table. The table moves to the starting position and the Kr/F excimer laser (248 nm) is focused through the first field of the photomask onto the metal laminate. Laser light passing through the clear areas of the photomask field ablates the metal from the metal laminate. Chromium coated areas of the photomask block the laser light and prevent ablation in those areas, resulting in a metallized sensor structure on the laminate film surface. The complete structure of the sensor traces may require additional ablation steps through various fields on the photomask.

Another method of preparing the described micro-electrode array is the use of flex circuit photolithography. Flex circuit photolithography methods are well known. Two general methods of fabricating flexible circuits include the "additive" method and the "subtractive" method. With the additive method, an IDA and associated circuitry can be built up on top of a non-conductive flexible substrate. With the subtractive method, a non-conductive flexible substrate can be laminated with a conductive material (e.g., a copper foil) and conductive material is patterned using conventional photolithographic and wet chemical etching techniques. Some conventional processing steps include cleaning a substrate or intermediate; depositing conductive materials onto a substrate, e.g., by vapor deposition, electrodeposition, or vacuum plasma sputtering; depositing non-conductive or processing materials onto a substrate such as a photoresist material; masking and developing a photoresist material in a pattern defining an electrode; and removing excess developed or non-developed materials such as photoresist materials or conductive materials, to leave behind a desired arrangement of electrically conductive and insulating materials.

According to one series of steps in flex circuit photolithography, a substrate is prepared by cleaning, and a conductive material can be applied as a film to the substrate. Preferred thicknesses of a conductive layer (e.g., a gold conductive layer) can be in the range from about 500 to 1000 angstroms. It may be desirable to include a seed layer such as titanium or chromium between the conductive layer and the substrate, to improve adhesion. A preferred conductive material can be gold, and a preferred method of application can be sputtering, which has been found to provide very good adhesion.

A photoresist material can be applied to the conductive layer. Such photoresist materials are commercially known and available, with one example being Riston® CM206, from duPont. The thickness of the photoresist can be chosen to advantageously affect the resolution of the feature sizes of the electrode components. Improved resolution generally provides for better quality arrays, with fewer failures. There has been found a 1:1 relationship between the resolution of the smallest feature size achievable, and the thickness of the dry film photoresist, with thinner photoresist films providing better resolution (a thickness of about 0.6 mils generally allows a feature spacing or width of 0.6 mils). Riston® CM206, in the form of a 0.6 mil thick roll of film, can be a preferred photoresist because it can be capable of resolving features, i.e., lines and spaces, on a lower micron scale, e.g.,

in the range of 0.6 mils (15 microns) or lower. A photoresist layer often requires heating. Riston® CM206 does not require prebaking. The material is a dry film photoresist and is applied to the gold substrate using a heated laminated roller system. Once laminated, the material is ready for processing (exposure to UV light, and development).

The laminated film can be cut to a convenient size, e.g., one foot by one foot, and a pattern defining a micro-electrode array can be cured or crosslinked. This can generally be accomplished by conventional methods, e.g., using a mask pattern and exposing the array pattern to ultraviolet light, crosslinking the photoresist in the pattern of the array. Unexposed, uncrosslinked, photoresist can be developed away using a developing agent, which will typically be particular to the photoresist composition (e.g., lithium carbonate is one developing agent; see the manufacturer's instructions). At the end of this step, the substrate will have an undisturbed layer of the conductive material coated thereon, with a photoresist design defining the pattern of the array laid out on the conductive layer. This allows for unprotected conductive material to be etched away using an etchant (e.g., KI/I_2), to produce the IDA pattern in the conductive material. The remaining photoresist can then be removed.

Once an array is prepared, e.g., by laser ablative methods, using laminated dry photoresist, spin coating, etching, or other techiniques, further processing of the micro-electrode array can be used to incorporate the array into a useful electronic device such as a biosensor. Preferably, additional materials can be disposed onto the array to form, for example, a spacer or insulating layer, optionally including a well or a microchannel or capillary. A well refers to a space over an array that defines the array. A microchannel or capillary more specifically refers to a space or channel that is defined over the array to allow the flow of a fluid over the array. The material used to define the microchannel or capillary can be any of a variety of materials useful insulating or spacing materials, sometimes referred to as "coverlay" materials, as well as other material useful for processing with the described fabrication methods. An example is Pyralux coverlay, and similar materials would also be useful.

Methods useful to place a microchannel or capillary onto the array include methods of mechanical lamination and mechanical removal of material to form a channel or capillary. One method would include a first step of mechanically "punching" (e.g., die punching) the coverlay material to cut away one or multiple portions of the material in the form of wells or channels, and then laminating the material to one or a number of sensors such that the channel is present over the array. Another method includes those types of methods generally referred to as "kiss die cutting" or "kiss cutting," which may be used to cut a well or channel in a coverlay layer, and then the coverlay material may be laminated onto the substrate with the well or channel over the array.

A different example that includes a die punching method is as follows. A spacer foil was prepared by coating an adhesive, Fastbond™ 30-NF Contact Adhesive to a wet thickness of 25 μm onto a 5 mil polyester film such as that sold under the trademark Melinex® S (DuPont Polyester films, Wilmington Del.) using a wire bar coater from Thomas Scientific of Swedesboro, N.J. The coated top foil was dried for 2 minutes at 50 C in a horizontal air flow oven. The dried adhesive on the sheet was covered with either silicon or teflon release liner. Capillary channels and electrode contact well patterns were kiss cut into the sheet using an Aristomat 1310 ditigal die cutting system (Aristo Graphic Systeme GmbH & Co., Hambrug Germany). The spacer sheet can then be registered and laminated to an ablated sheet of sensor traces, as described above. Channels and electrode contact wells can also be produced using die punching processes in a similar fashion.

Another specific method by which to dispose a capillary or microchannel onto a micro-electrode array would be by methods of flex circuit photolithography. Accordingly, a photoimageable coverlay material such as Vacrel 8140®, (a dry film coverlay can be preferred) can be vacuum laminated onto the gold/plastic laminate. Multiple layers of various chosen thicknesses can be added to control the depth of the capillary chamber (see infra). The sheet can be exposed to ultraviolet light through a mask pattern to define capillary chambers. The exposed laminated sheet is developed by conventional methods, e.g., using 1% K_2CO_3, to remove crosslinked photopolymer coverlay material and leave behind components of a capillary. The sheet is generally thereafter cured, e.g., at 160 C for 1 hour.

In fabricating the capillary, the depth of the chamber can be controlled by choosing the coverlay material or materials used, according to thickness. Vacrel 8140® (film has a thickness of 2, 3, or 4 mil (100 μm). Pyralux PC® 1000, 1500, have 2000 have maximum thicknesses of 2.5 mils (63.5 μm), so double layer lamination gives a chamber depth of 127 μm. Pyralux 1010 has a thickness of 1 mil or 25.4 μm. Capillaries with depths of greater than or equal to 100 μm have been found to allow fast fill of blood with hematocrits from 20 to 70% to reliably flow into the chamber. Capillary depths of less than 100 microns to 25 microns can be used for other biological fluids such as serum, plasma, interstitial fluid, and the like.

A chemical coating may also be disposed onto the array. First, however, it may be beneficial to clean the sensors. By one cleaning method, a sheet of sensors as described can be plasma cleaned in a Branson/IPC Plasma Cleaner according to steps such as the following: (1) O_2 for 1 minute at 800 watts; (2) O_2/Argon(Ar) (70/30) for 3 minutes at 220 watts; (3) Ar for 2 minutes at 150 watts.

A chemical coating, as described, may be dispensed onto the array, e.g., into each capillary chamber and over the interdigitated arrays, by known methods. The method of dispensing is preferably capable of reproducibly and consistently delivering very small volumes of a chemical composition, onto the array—e.g., volumes in the range of hundreds of nanoliters, e.g., 625 nanoliters. As an example, such a coating may be dispensed using known syringe and metering techniques and apparatuses, including dispensing systems sold under the trade name Microdot (from Astro Dispense Systems, a DCI Company of Franklin, Mass. 02038-9908) and systems sold by BioDot Inc., Irvine, Calif. The coatings may then be dried of solvent. The inlet ports are opened, and a top foil coated with a hydrophilic adhesive is applied over the capillary chamber using heat and pressure to form the completed three-dimensional sensor structure.

The top foil can be any continuous film capable of defining one side of the capillary, and preferably being capable of appropriate processing, e.g., as described herein. Exemplary materials for the foil can include plastic films such as polyethylene naphthalate (PEN), film type Kadalex 1000, 7 mil thick.

Any of a variety of hydrophilic adhesives can be used to bond the top foil to the sensor. Two part thermoset adhesives such as polyurethane mixtures and isocyanate mixtures can be used, e.g., 38-8668 (polyurethane) and 38-8569 (isocyanate) (95:5 wt./wt.) from National Starch and Chemical Co. of Bridgwater N.J., or, a two part epoxy systems such as that

sold under the trademark Scotch Weld™ 2216 B/A (3M Adhesive Div., St. Paul Minn.), as well as contact adhesives such as that sold under the trademark Fastbond™ 30-NF Contact Adhesive, provided that they exhibit acceptable sealing properties to the crosslinked coverlay surface. A preferred adhesive was found to be a mixture of Fastbond™ 30-NF Contact Adhesive and the surfactant Triton™ X-100 (Union Carbide, Danbury Conn.), 93%:7% wt./wt.

EXAMPLES

The following describes a process useful for preparing a sensor according to the invention, comprising an interdigitated array disposed on a flexible substrate. According to the method, a gold film can be deposited onto 7 mil thick Kaladex® film using a planar DC magnetron sputtering process and equipment, from Techni-Met Inc., Windsor, Conn. The thickness of the gold film can range from 30 to 200 nm, with a preferred thickness being 100 nm. Seed layers of chromium or titanium can be sputtered between the plastic film and the gold to promote better adhesion of the gold to the plastic substrate, however, gold layers sputtered directly onto the plastic film can exhibit sufficient adhesion.

The interdigitated array and connectors can be fabricated using batch photolithography processes common to the flex circuit industry. Electrodes with combinations of finger width and spacing between fingers in the range of 21 to 50 μm were easily fabricated using these processes. A preferred configuration of the array was 21 total fingers (10 working electrode fingers and 11 counter electrode fingers), with finger dimensions of 25 microns (width) by 1 millimeter (length), with 21 micron spacing between the fingers.

After the gold was applied to the flexible substrate, a dry film photopolymer resist was laminated to the gold/plastic film. A dry film resist such as that sold under the trademark Riston® CM206 (duPont) was preferred. The Riston® CM206 photoresist was first wet laminated onto the gold surface of 12"×12" gold/plastic panels using a HRL-24 hot roll laminator (from duPont). The sealing temperature and lamination speed were 105° C. and 1 meter per minute. The laminated panel was placed in a Tamarack model 152R exposure system, from Tamarack Scientific Co., Inc., Anaheim, Calif. The release liner was removed from the top surface of the photoresist. A glass/emulsion photomask of the IDA configuration was produced by Advance Reproductions Corporation, North Andover, Mass. The emulsion side of the mask was treated with an antistick coating (Tribofilm Research Inc., Raleigh, N.C.), and was placed directly onto the photoresist surface of the panel. The laminated panel was exposed to ultraviolet light of 365 nm through the photomask using an exposure energy of 60 mJ/cm^2. Exposed photoresist was stripped from the panel in a rotary vertical lab processor (VLP-20), Circuit Chemistry Equipment, Golden Valley, Minn., using 1 % potassium carbonate, at room temperature, for 30 seconds using a nozzle pressure of 34 psi. Exposed gold on the sheet was then stripped using an etch bath containing a solution of: 4 parts I_2:1 part KI:40 parts water vol./vol.; and 0.04 gram Fluorad™ fluorochemical surfactant FC99, (3M, St. Paul, Minn.) per 100 gram solution, added to the bath to ensure wetting of the photoresist. Air was bubbled through the bath during the etch process to obtain uniform agitation of the bath mixture. The panel was rinsed with deionized water and residual Riston® CM206 was removed in a 3% KOH bath.

Sensor chambers were fabricated using dry film photoimageable coverlay materials such as that sold under the trademark Vacrel® 8140 (duPont) or Pyralux® PC series (duPont). The chamber dimensions can be accurately defined by flex circuit photolithography. Depth of the chamber was controlled by the thickness of the coverlay materials used, and whether single or multiple layers of the coverlay dry film were used. A preferred chamber depth was 125 microns (5 mil). This chamber depth was achieved by sequential lamination of different coverlay materials as follows: three mil thick Vacrel® 8130 was first laminated to the electrode side of the substrate using a HRL-24 (duPont) heated roll laminator at room temperature, using a roller speed of 1 meter per minute. The electrode panel was then vacuum laminated in a DVL-24 vacuum laminator (duPont) using settings of 120° F., 30 second vacuum dwell, and a 4 second pressure to remove entrapped air between the coverlay film and the electrode substrate. Two mil thick Vacrel 8120 was laminated next to the Vacrel® 8130 surface using the HRL-24 at room temperature, with a roller speed of 1 meter/min. The panel was then vacuum laminated again in the DVL-24 vacuum laminator using a 30 second vacuum dwell, 4 second pressure, to remove entrapped air between the two coverlay films.

The laminated electrode sheet was placed in the Tamarack 152R system and was exposed to ultraviolet light at 365 nm through the photomask for 22 seconds using an exposure intensity of 17 mW/cm^2. The artwork for the capillary chamber was a 1 millimeter by 4 millimeter rectangle centered over the electrode finger array and starting 1 millimeter below the fingers. The exposed coverlay was stripped from the panel to reveal the sensor chamber rectangle using the VLP-20 Circuit Chemistry Equipment) in 1% K_2CO_3, at 140° F., for 75 seconds using a nozzle pressure of 34 psi. The developed laminate structure was rinsed in deionized water, and then cured at 160° C. for 1 hour to thermally crosslink the coverlay material. This completed the construction of the sensor base.

The panel of the base sensors was plasma cleaned to remove residual photoresist and coverlay material from the exposed gold surface of the interdigitated array structure. The panel was placed in a barrel etcher, a Barnstead/IPC model P2100 from Metroline/IPC of Corona, Calif. The panel was first exposed to an oxygen plasma for 1 minute at 800 watts and 1.1 torr pressure to oxidize the panel surface. It was then etched in an oxygen/argon plasma mixture (70/30 vol./vol.) for 3 minutes, at 225 watts and 1.5 torr pressure, and was finally stripped in an argon plasma for 2 minutes, at 150 watts and 2 torr pressure.

The chemical coating was formulated for measurement of d-glucose in a human blood sample. The chemical coating was reactive with the sample in a manner effective to generate an electrical output signal indicative of the level of glucose in the sample. The coating included a mediator, enzymes, and a cofactor. The coating further comprised film forming agents and detergents conferring durability and providing hydrophilicity. The ingredients are listed in Table 1; unless stated otherwise, all concentrations refer to the concentration of a given substance in a wet-coating, prior to the deposition and drying of the coating onto the array.

The chemical coating was formulated from several submixtures of components. A first mixture contained glycerophosphate buffer, from ICN Biomedicals Inc. Aurora, Ohio; Medium Viscosity Alginic acid, from Sigma Chemical Co., St. Louis, Mo.; Natrosol 250M, from Hercules Inc., Wilmington, Del.; and Triton® X-100, from Union Carbide, Danbury Conn. These components were added to a volume of distilled water sufficient to make a 250 gram solution of the buffer/polymer/surfactant (see Table 1). The solution was mixed overnight to allow complete hydration of the

Natrosol and Alginic acid. The pH of the completed solution was adjusted to 6.9 to 7.0 with concentrated hydrochloric acid. This solution is known hereinafter as "Solution A."

A second solution prepared was a concentrated enzyme/cofactor matrix. 8.2 milligrams pyrrolo-quinoline-quinone (PQQ), Fluka, Milwaukee, Wis., was added to 25.85 grams of Solution A. The resulting mixture was sonicated until the PQQ was completely in solution. 1.1394 grams of the enzyme, Glucose-De-oxidoreductase (GlucDor), from Roche Molecular Biochemicals, Indianapolis, Ind., was added to the solution. The final mixture was rocked for 2 hours to allow formation of the GlucDor/PQQ holoenzyme. The completed solution will be referred to as "Solution B."

Potassium ferricyanide was added to the composition as follows: 4.4173 grams of potassium ferricyanide, from J. T. Baker, Phillipsburg, N.J., was added to 70.58 grams of Solution A. The resulting solution was mixed until the ferricyanide was completely in solution. The completed solution will be referred to as "Solution C."

The final coating composition was completed by combining 63 grams of Solution C to 25 grams Solution B. This composition was rocked in the dark for 1 hour to thoroughly mix.

TABLE 1

Formulation per 100 grams of coating

Component	Concentration/ activity	Wet mass (g)	Dry mass/sensor (mg)
Distilled Water		88.487	
Disodium Glycerophosphate	150 mM pH 6.98	4.359	0.0287
Trehalose	1% wt/wt	1.000	0.0066
Natrosol	0.3% wt/wt	0.300	0.002
Alginic acid Medium viscosity	0.4% wt/wt	0.400	0.0026
Triton X-100	0.025% wt/wt	0.025	0.00016
Pyrrolo-quinoline-Quinone (PQQ)	0.261 mM	0.0082	5.3382 × .10 − 5
GlucDor Enzyme	2034 u/mg	1.1394	0.0075 15.23 (units)
Potassium Ferricyanide	137 mM	4.2814	0.0281

A preferred method for applying the chemistry matrix to the sensor chamber (IDA) is a discrete dispense of 500 nanoliters of the coating solution into the 1 millimeter×4 millimeter chamber using a microdispensing system such as that sold under the trademark of BioJet Quanti3000®, BioDot Inc., Irvine, Calif. The coating covered both the working and counter electrodes of the IDA. The coating was dried for 1.5 minutes at 45° C. in a horizontal air flow oven, VWR Scientific Products, Chicago Ill.

The hydrophilic top foil was prepared by coating an adhesive mixture (e.g., a mixture of Fastbond™ 30-NF Contact Adhesive and the surfactant Triton™ X-100 (Union Carbide, Danbury Conn.), 93%:7% wt/wt.) to a wet thickness of 25 μm onto 5 mil polyester film such as that sold under the trademark Melinex® "S" (duPont Polyester Films, Wilmington Del.) using a wire bar coater from Thomas Scientific, Swedesboro, N.J. The coated top foil was dried for 2 minutes at 50° C. in a horizontal air flow oven (VWR Scientific Products). The capillary chamber was opened by cutting 1 millimeter in from the front edge of the capillary chamber with a pair of scissors. The dried coated top foil was applied to the sensor, allowing approximately a 0.5 mm space between the back edge of the chamber and the edge of the top foil as an air vent. The top foil was sealed to the sensor surface using a 5 ton press with a heated top platen, at 81° C., 60 psi for 5 seconds. The panel of completed sensors was cut into individual sensors and stored desiccated at 8% RH until tested.

The sensors were evaluated using chronoamperometry electrochemical techniques on test stands such as that sold under the trademark of BAS® 100W Electrochemical Workstation, Bioanalytical Systems, Inc. West Lafayette, Ind. The preferred electrochemical test stand used in the evaluation of the electrodes was a dedicated test stand for DC chronoamperometric current measurement for assay potentials from ±1 volt.

The sensors may be used to determine the concentration of an analyte, such as glucose, in a fluid sample by performing the following steps:

Set up the test stand parameters:

- In accordance with a "drop detect" system, an initial potential difference is established between the working and counter electrodes—300 mV (millivolts)—to start timing of the analysis sequence. Current response to this potential is triggered by contact of the array with a fluid sample.
- The initial current response upon application of the test solution to the sensor chamber is generally greater than 0.4 microamps.
- The time (delay period) between the threshold trigger and re-application of the 300 mV potential difference (assay potential) is generally 3 seconds.
- The assay period, after re-application of the 300 mV potential difference between the working and counter electrodes of the sensor is generally 9 seconds.

In more detail:

- Insert the sensor into the test stand connection. Apply approximately 0.3 uL of a fluid sample to the opening of the capillary chamber. Fluid will flow into the chamber by capillary action covering the chemical coating applied to the working and counter electrodes. The threshold current will be triggered when the sample fluid covers the nearest working and counter electrode fingers. Once triggered, the potential difference will go to open circuit for a 3 seconds, during the delay period.
- During the delay period, reaction will occur between the reactants (analyte, enzyme/cofactor, and the oxidized form of the mediator), resulting in reduction of the mediator.
- The 300 mV assay potential difference is re-applied between the electrodes after the 3 second delay. This causes electro-oxidation of the reduced mediator at the surface of the working electrode.
- The current/time reaction profiles of the assay show a characteristic pseudo-steady-state current/time plateau starting 0.5 to 1.5 seconds after re-application of the 300 mV assay potential to the sensor. Currents at fixed assay period points chosen in this plateau region were proportional to the concentration of analyte in the sample fluid. Assay endpoints were chosen in such a manner give a linear dose response for glucose concentrations from 0 to 600 mg/dL. See FIG. **7**.

The invention claimed is:

1. A method of determining the concentration of glucose in a blood sample, comprising:

providing a disposable biosensor test strip including a capillary chamber having a depth suitable for capillary flow of blood and holding a volume of less than about 1.0 μl of the blood sample, a working electrode and a

counter or reference electrode disposed within the capillary chamber, and a reagent proximal to or in contact with at least the working electrode, the reagent including an enzyme and a mediator, the reagent reacting with glucose to produce an electroactive reaction product;

applying a blood sample containing glucose into the capillary chamber, the capillary chamber directing capillary flow of the blood sample into contact with the reagent to cause the blood sample to at least partially solubilize or hydrate the reagent;

detecting the blood sample in the capillary chamber;

electrooxidizing or electroreducing the electroactive reaction product at the working electrode; and

within 10 seconds after said detecting, determining and providing a readout of the glucose concentration in the blood sample, said determining comprising correlating the electrooxidized or electroreduced electroactive reaction product to the concentration of glucose in the blood sample.

2. The method of claim **1** in which said detecting comprises applying a dose-detect potential between the working and counter or reference electrodes, and recognizing a rise in current as an indication that the sample has been supplied to the capillary chamber.

3. The method of claim **1** in which the test strip includes a vent communicating with the capillary chamber to facilitate flow of the sample into the capillary chamber.

4. The method of claim **1** in which said providing comprises providing the reagent in a sufficiently small amount as to be solubilized or hydrated in a time sufficient to allow said determining and providing a readout of the glucose concentration in the sample within 10 seconds after said detecting.

5. The method of claim **1** in which said providing comprises providing a test strip including a bottom substrate, a spacing layer, and a top substrate, the spacing layer having an opening corresponding to the capillary chamber, the spacing layer substantially defining the depth of the capillary chamber.

6. The method of claim **5** in which the test strip is elongated with two opposed sides, the spacing layer comprising spaced-apart first and second portions defining a capillary chamber extending between and opening at the two opposed sides.

7. The method of claim **1** in which said providing comprises providing a mediator in its oxidized form.

8. The method of claim **7** in which the mediator reacts sufficiently rapidly as to allow said determining and providing a readout of the glucose concentration in the sample within 10 seconds after said detecting.

9. The method of claim **8** in which said providing comprises providing the reagent in a sufficiently small amount as to be solubilized or hydrated in a time sufficient to allow said determining and providing a readout of the glucose concentration in the sample within 10 seconds after said detecting.

10. The method of claim **9** in which the capillary chamber holds a volume of less than about 0.4 μl.

11. The method of claim **10** in which the capillary chamber holds a volume of between about 0.25 μl and about 0.4 μl.

12. The method of claim **11** in which said detecting comprises applying a dose-detect potential between the working and counter or reference electrodes, and recognizing a rise in current as an indication that the sample has been supplied to the capillary chamber.

13. The method of claim **12** in which the test strip includes a vent communicating with the capillary chamber to facilitate flow of the sample into the capillary chamber.

14. The method of claim **13** in which said providing comprises providing a test strip including a bottom substrate, a spacing layer, and a top substrate, the spacing layer having an opening corresponding to the capillary chamber, the spacing layer substantially defining the depth of the capillary chamber.

15. The method of claim **14** in which the test strip is elongated with two opposed sides, the spacing layer comprising spaced-apart first and second portions defining a capillary chamber extending between and opening at the two opposed sides.

16. The method of claim **1** in which the capillary chamber holds a volume of less than about 0.4 μl.

17. The method of claim **16** in which the capillary chamber holds a volume of between about 0.25 μl and about 0.4 μl.

18. The method of claim **1** in which said electroactive reaction product is capable of being electrooxidized or electroreduced at the working electrode, said determining comprising measuring the amount of electroactive reaction product electrooxidized or electroreduced, and correlating the amount of electrooxidized or electroreduced electroactive reaction product to the concentration of glucose in the blood sample.

19. The method of claim **1** in which said capillary chamber has a depth of 25–200 μm.

20. The method of claim **1** including automatically operating the test strip and timing the reaction and analysis of the blood sample to detect the blood sample in the capillary chamber, to electrooxidize the electroactive reaction product, and to determine and provide a readout of the glucose concentration within 10 seconds of said detecting.

21. The method of claim **1** in which the test strip comprises a counter electrode, and in which the reagent is located proximal to or in contact with the working and counter electrodes.

22. The method of claim **1** in which the capillary chamber holds a volume of about 600 nL.

23. The method of claim **22** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

24. The method of claim **1** in which the capillary chamber holds a volume of between 0.25 μl and 0.4 μl.

25. The method of claim **24** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

26. The method of claim **24** in which the capillary chamber holds a volume of about 400 nL.

27. The method of claim **26** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

28. The method of claim **24** in which the capillary chamber holds a volume of about 300 nL.

29. The method of claim **28** comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

30. The method of claim **1** comprising determining and providing a readout of the glucose concentration within about 8 seconds of said detecting.

31. The method of claim **30** comprising determining and providing a readout of the glucose concentration about 3.5 to about 8 seconds after said detecting.

32. The method of claim 31 comprising determining and providing a readout of the glucose concentration within about 5 seconds of said detecting.

33. The method of claim 32 comprising determining and providing a readout of the glucose concentration within about 4 seconds of said detecting.

34. The method of claim 31 comprising determining and providing a readout of the glucose concentration about 5 seconds after said detecting.

35. The method of claim 31 comprising determining and providing a readout of the glucose concentration about 4 seconds after said detecting.

36. A method of determining the concentration of glucose in a blood sample, comprising:

providing a disposable biosensor test strip including a capillary chamber having a depth suitable for capillary flow of blood and holding a volume of less than about 1.0 μl of the blood sample, a working electrode and a counter or reference electrode disposed within the capillary chamber, and a reagent proximal to or in contact with at least the working electrode, the reagent including an enzyme and a mediator, the reagent reacting with glucose to produce an electroactive reaction product;

applying a blood sample containing glucose into the capillary chamber, the capillary chamber directing capillary flow of the blood sample into contact with the reagent to cause the blood sample to at least partially solubilize or hydrate the reagent;

detecting the blood sample in the capillary chamber;

electrooxidizing the electroactive reaction product at the working electrode; and

within 10 seconds after said detecting, determining and providing a readout of the glucose concentration in the blood sample, said determining comprising correlating the electrooxidized electroactive reaction product to the concentration of glucose in the blood sample.

37. The method of claim 36 in which the capillary chamber holds a volume of about 600 nL.

38. The method of claim 37 comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

39. The method of claim 36 in which the capillary chamber holds a volume of between 0.25 μl and 0.4 μl.

40. The method of claim 39 comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

41. The method of claim 39 in which the capillary chamber holds a volume of about 400 nL.

42. The method of claim 41 comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

43. The method of claim 39 in which the capillary chamber holds a volume of about 300 nL.

44. The method of claim 43 comprising determining and providing a readout of the glucose concentration within about 5 seconds after said detecting.

45. The method of claim 36 comprising determining and providing a readout of the glucose concentration within about 8 seconds of said detecting.

46. The method of claim 45 comprising determining and providing a readout of the glucose concentration about 3.5 to about 8 seconds after said detecting.

47. The method of claim 46 comprising determining and providing a readout of the glucose concentration within about 5 seconds of said detecting.

48. The method of claim 47 comprising determining and providing a readout of the glucose concentration within about 4 seconds of said detecting.

49. The method of claim 46 comprising determining and providing a readout of the glucose concentration about 5 seconds after said detecting.

50. The method of claim 46 comprising determining and providing a readout of the glucose concentration about 4 seconds after said detecting.

51. The method of claim 36 in which the reagent is dry, and the capillary chamber directs capillary flow of the blood sample into contact with the dry reagent to cause the blood sample to at least partially solubilize or hydrate the dry reagent.

52. The method of claim 51 in which the dry reagent comprises a reagent that is applied wet and dried of solvent.

53. The method of claim 36 in which the capillary chamber has a depth of 25-200 μm.

54. The method of claim 36 in which said providing comprises providing the reagent in a sufficiently small amount as to be solubilized or hydrated in a time sufficiently fast to allow said determining and providing a readout of the glucose concentration in the blood sample within 10 seconds of said detecting.

55. The method of claim 54 in which the mediator reacts sufficiently rapidly as to allow determining and providing a readout of the glucose concentration in the blood sample within 10 seconds of said detecting.

56. The method of claim 55 in which the mediator is readily-reversible.

57. The method of claim 36 including automatically operating the test strip and timing the reaction and analysis of the blood sample to detect the blood sample in the capillary chamber, to electrooxidize the electroactive reaction product, and to detennine and provide a readout of the glucose concentration within 10 seconds of said detecting.

58. The method of claim 57 in which said automatically operating comprises connecting the test strip to an external testing apparatus prior to said detecting, the testing apparatus automatically detecting the blood sample in the capillary chamber, electrooxidizing the electroactive reaction product, determining the glucose concentration, and providing a readout of the glucose concentration within 10 seconds of said detecting.

59. The method of claim 36 in which the working electrode and the counter or reference electrode are coplanar.

60. The method of claim 36 comprising measuring the current and correlating the measured current to the glucose concentration.

61. The method of claim 36 in which said detecting comprises applying a drop-detect potential across the working and counter or reference electrodes.

62. The method of claim 61 in which said detecting comprises applying a drop-detect potential across the working and counter or reference electrodes prior to and separate from said determining.

63. The method of claim 62 in which said detecting comprises applying a drop-detect potential across the working and counter or reference electrodes and recognizing a rise in current as an indication that the blood sample has been applied into the capillary chamber.

64. The method of claim **62** which includes reapplying a potential across the working and counter or reference electrodes, after a delay period during which no potential is applied, to electrooxidize the electroactive reaction product at the working electrode.

65. The method of claim **36** in which said providing comprises providing a test strip including a bottom substrate, a spacing layer, and a top substrate, the spacing layer having an opening corresponding to the capillary chamber, the spacing layer substantially defining the depth of the capillary chamber.

66. The method of claim **36** in which said reacting produces a reduced form of the mediator.

67. The method of claim **36** comprising measuring the amount of the electrooxidized electroactive reaction product and correlating the amount to the concentration of glucose in the blood sample.

68. The method of claim **36** comprising applying a potential of 100-500 mV across the working electrode and the counter or reference electrodes.

69. The method of claim **36** in which the test strip comprises a counter electrode, and in which the reagent is located proximal to or in contact with the working and counter electrodes.

* * * * *

US007276147C1

(12) EX PARTE REEXAMINATION CERTIFICATE (6677th)

United States Patent

Wilsey

(10) Number: US 7,276,147 C1

(45) Certificate Issued: Feb. 24, 2009

(54) **METHOD FOR DETERMINING THE CONCENTRATION OF AN ANALYTE IN A LIQUID SAMPLE USING SMALL VOLUME SAMPLES AND FAST TEST TIMES**

(75) Inventor: **Christopher D. Wilsey**, Carmel, IN (US)

(73) Assignee: **Corange International Limited**, Hamilton (BM)

Reexamination Request:
No. 90/010,080, Dec. 14, 2007

Reexamination Certificate for:

Patent No.:	**7,276,147**
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Appl. No.:	**10/382,322**
Filed:	**Mar. 5, 2003**

(*) Notice: This patent is subject to a terminal disclaimer.

Related U.S. Application Data

(63) Continuation of application No. 10/264,785, filed on Oct. 4, 2002, now abandoned

(60) Provisional application No. 60/332,411, filed on Nov. 16, 2001.

(51) **Int. Cl.**
G01N 27/327 (2006.01)

(52) **U.S. Cl.** **205/792**; 205/777.5; 204/403.4

(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

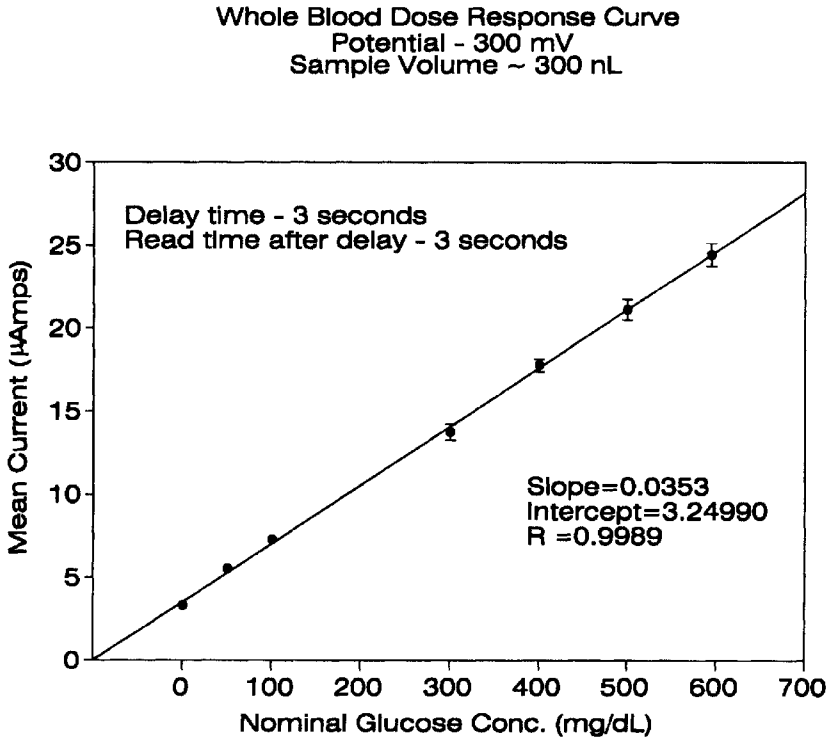
WO WO 00/20626 A1 4/2000

Primary Examiner—Gary L Kunz

(57) **ABSTRACT**

Analytes in a liquid sample are determined by methods utilizing sample volumes of less than about 1.5 µl and test times within ten seconds. The methods are preferably performed using small test strips including a sample receiving chamber filled with the sample by capillary action.

AMENDED



AMENDED

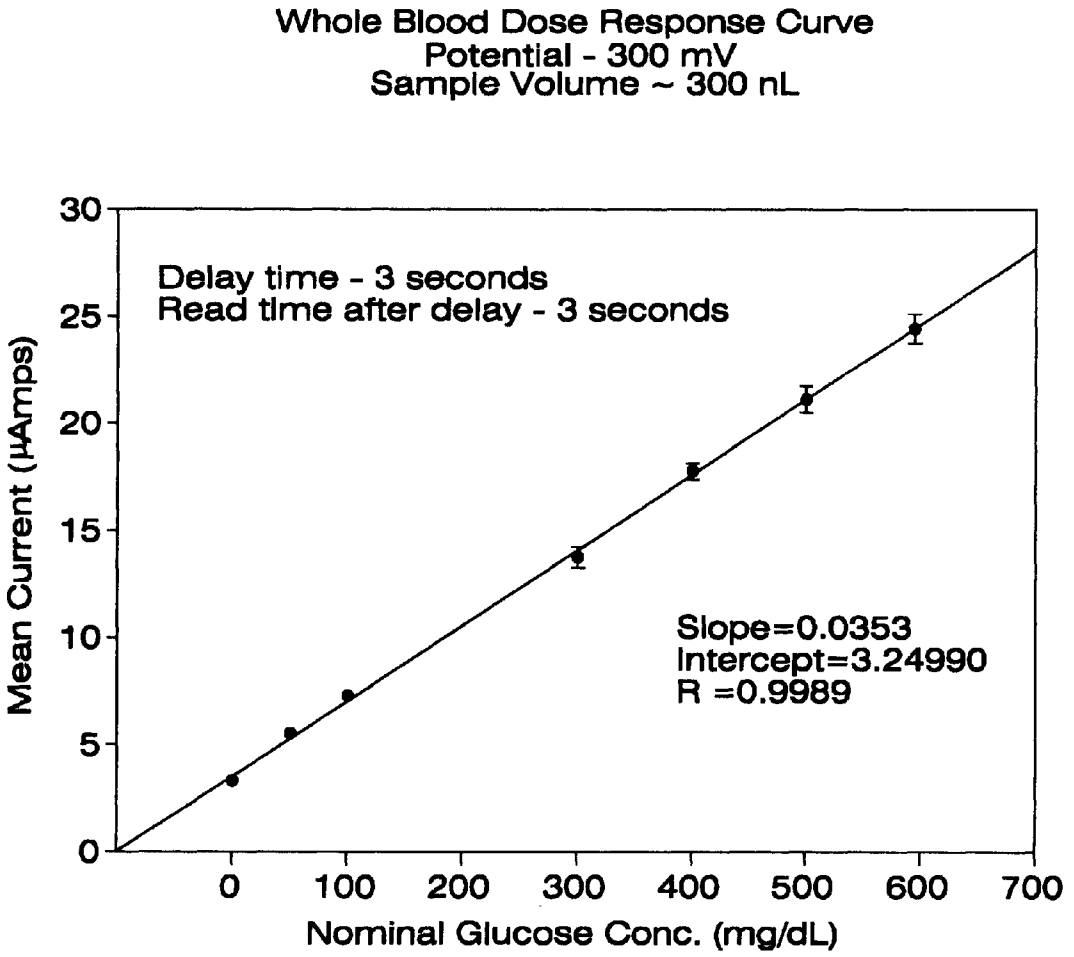


Fig. 7

EX PARTE REEXAMINATION CERTIFICATE ISSUED UNDER 35 U.S.C. 307

THE PATENT IS HEREBY AMENDED AS INDICATED BELOW.

Matter enclosed in heavy brackets [ ] appeared in the patent, but has been deleted and is no longer a part of the patent; matter printed in italics indicates additions made to the patent.

ONLY THOSE PARAGRAPHS OF THE SPECIFICATION AFFECTED BY AMENDMENT ARE PRINTED HEREIN.

Column 18, lines 20–32:

In fabricating the capillary, the depth of the chamber can be controlled by choosing the coverlay material or materials used, according to thickness. Vacrel 8140® **[(]** film has a thickness of 2, 3, or 4 mil (100 μm). Pyralux PC® 1000, 1500, have 2000 have maximum thicknesses of 2.5 mils (63.5 μm), so double layer lamination gives a chamber depth of 127 μm. Pyralux 1010 has a thickness of 1 mil or 25.4 μm. Capillaries with depths of greater than or equal to 100 μm have been found to allow fast fill of blood with hematocrits from 20 to 70% to reliably flow into the chamber. Capillary depths of less than 100 microns to 25 microns can be used for other biological fluids such as serum, plasma, interstitial fluid, and the like.

THE DRAWING FIGURES HAVE BEEN CHANGED AS FOLLOWS:

(uAmps) to (μAmps) in FIG. **7**.

AS A RESULT OF REEXAMINATION, IT HAS BEEN DETERMINED THAT:

The patentability of claims **1**–**56** and **59**–**69** is confirmed.

Claim **57** is determined to be patentable as amended.

Claim **58**, dependent on an amended claim, is determined to be patentable.

57. The method of claim **36** including automatically operating the test strip and timing the reaction and analysis of the blood sample to detect the blood sample in the capillary chamber, to electrooxidize the electroactive reaction product, and to **[**detennine**]** *determine* and provide a readout of the glucose concentration within 10 seconds of said detecting.

* * * * *

CERTIFICATE OF SERVICE

I hereby certify that, on April 21, 2015, I electronically filed the a copy of BRIEF OF PLAINTIFFS-APPELLANTS ROCHE DIAGNOSTICS OPERATIONS, INC. AND CORANGE INTERNATIONAL LIMITED with the Clerk of Court using the CM/ECF System upon all counsel of record.

I further certify that, upon acceptance and request from the Court, the required paper copies of the foregoing will be deposited with Federal Express Service for delivery to the Clerk, UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT, 717 Madison Place, N.W., Washington, D.C. 20439.

Dated: April 21, 2015

/s/ Paula S. Fritsch
Paula S. Fritsch
Attorney for Plaintiffs-Appellants
Roche Diagnostics Operations, Inc.
and Corange International Limited

CERTIFICATE OF COMPLIANCE

WITH TYPE-VOLUME LIMITATION, TYPE FACE REQUIREMENTS,

AND TYPE STYLE REQUIREMENTS

This brief complies with the type-volume limitation of Federal Rule of Appellate Procedure 32(a)(7)(B), because the brief contains 13,876 words, as reported by the word count function in the Microsoft Word 2010 word processing program, excluding the parts of the brief exempted by Federal Rule of Appellate Procedure 32(a)(7)(B)(iii) and Federal Circuit Rule 32(b).

This brief complies with the typeface requirements of Federal Rule of Appellate Procedure 32(a)(5) or Federal Rule of Appellate Procedure 28.1(e) and the type style requirements of Federal Rule of Appellate Procedure 32(a)(6) because the brief has been prepared in a proportionally spaced typeface, 14 point Times New Roman, using the Microsoft Word 2010 word processing program.

Dated: April 21, 2015

/s/ Paula S. Fritsch
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Roche Diagnostics Operations, Inc.
and Corange International Limited